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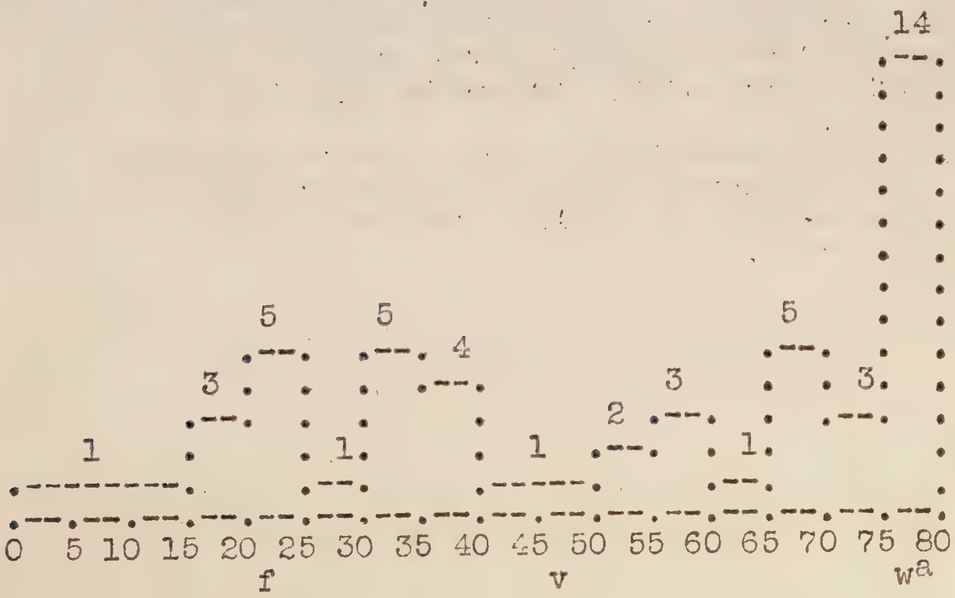
NOTES AND NEWS

Research items

Effect of ether on gene changes. S. Gershenson - In 1930 a large experiment with D.mel. was carried out with an aim of producing gene-mutations by means of etherization. Apricot larvae 2 and 4 days old were submitted to a sub-lethal dose of ether vapor (40-48 minutes in an atmosphere saturated with ether vapor at 16°C). Many variations were recorded in the flies which developed from the treated larvae (mostly bristle characters). 1200 x-chromosomes from 96 males studied (ClB method) gave a negative result (2 lethals) (U).

A case of ClB reinversion. S. Gershenson - In 1931 a sc^1 t v sl B D.melanogaster male was obtained from a female of the structure ClB/sc^3 v. Study of the chromosome showed that it not only lost its lethal action, but also was reinverted, the new breaks being probably precisely in the same points as the breaks of the ClB inversion. Two explanations are possible: either the lethal action of the ClB chromosome is due to a position effect, or the reinversion occurred simultaneously with a double crossing-over in the left non-inverted part of the ClB chromosome (which must then have carried the lethal). Contamination excluded. A semi-lethal was present in the reinverted chromosome, located near sl (U)

Distribution of x-chromosome lethals. S. Gershenson - In 1931 a study of the distribution of x-ray lethals in the sc^8 w^a In X of D.melanogaster was carried out (in collaboration with N.I. Shapiro and E.J. Borissenko). The following picture was obtained (51 lethal located) (U):



The manifestation of dominants in the triploid. Jack Schultz - Most of the dominants available in D.melanogaster have been observed in the triploid, in the course of a series of experiments concerned with the effects of upsets of genic balance on dominance relations. The following are easily classified when present in single dose in the triploid: Bar, Beadex^2, Bristle, Curly, Deformed, Dichaete, Hairless, Hairy-wing, Jammed, Lobe^2, Moire, Stubble. Those which are almost completely suppressed include: Delta, Gull, all Minutes, Notch, Plexate, Plum, Star. It may be noted that in no case is the manifestation as extreme in the triploid as it is in the diploid and many of the first group show a marked diminution of the effect.

A few of these dominants have been studied in double dose. The Minutes and Moiré do not survive. The following survive, manifesting the dominant characters in extreme form: Delta, Dichaete, Gull, Hairless, Plexate, Plum, Stubble.

Inversions in the x-chromosome of D.melanogaster - A. H. Sturtevant and G. W. Beadle - As is well known, there exist many different inversions in the X. When two of these are put in the same female, single crossovers occur within the common inverted region, and in several combinations viable crossover offspring are produced. The crossover chromosomes carry net deficiencies and/or duplications for the regions at the end-points of the inversions. These (especially the deficiencies) make it possible to determine the end-points with a precision limited only by the number of recessive mutations whose loci are already accurately mapped adjacent to the breaks.

The nomenclature of the inversions is now in a chaotic state; and becomes intolerably confusing when one begins dealing with crossovers between different inversions. We are using the following scheme: Each inversion is given an arbitrary letter; the sequence in ClB is referred to as "In B", that in y^4 as "In Y". Then, for each pair of inversions, two single crossovers are theoretically possible; these are described by the use of both letters concerned. In the case of the two referred to, the crossover that has the left end of ClB and the right end of y^4 is called "B-Y"; that with the left end of y^4 and the right of ClB (which has not been obtained) would be "Y-B".

The following inversions in the X have been studied in this laboratory. The end-points are given as accurately as our present data allow - they are being determined still more closely in most cases.

Current designation	New equivalent	Position of left inversion-point	Position of rt inversion-point
ClB	In B	Between ec and bi	Between sy and fu
dl-49	In D	No new data(near rg)	No new data(fw-g)
sc^8	In E	Between ac and sc	To rt of bb
y sc^4	In F	Between ac and rst	Between cr and bb
bb-deficiency*	In O	Between rb and rg	To rt of cr
roughest	In R	Uncertain	Uncertain
sc^7	In S	Unknown	Between fa and sn
y^4	In Y	Between y and ac	Between fu and cr

*Of Dobzhansky, not of Gershenson. The latter is "F-E" on our terminology.

In D and In S have not given single crossovers in any of our experiments. In R is a long inversion, which gives crossovers with several of the others; it is, however, complicated by the presence of a 1-3 translocation, with probably at least three points of breakage in X. The analysis is still incomplete.

The following crossovers have been obtained and studied: B-O, B-Y, E-F, E-Y, F-E, F-Y, O-Y, R-E, R-F, R-O, Y-E and Y-F. Others can presumably be produced, though several of them (such as Y-B) are known to be inviable.

These studies are being continued, with the object of attacking problems concerning crossing-over, disjunction, and the somatic effects of duplications and deficiencies.

Breakage point in x-chromosome for Blond-translocation (Tl-2). M. Demerec - A certain proportion of offspring from crosses with Blond are deficient for the yellow end of the x-chromosome from the point where the breakage occurred to the end of the chromosome. These flies have minute characteristics. In test made with y, ac, br and pn yellow, achaete and broad showed in minute flies but prune did not show. This indicates that pn is not included in the translocated piece and that y ac and br are included. The breakage point, therefore, is between br and pn.

Intersexes of D.virilis. G. A. Lebedeff - Out of four lines of flies producing morphologically different types of intersexes (Amer.Nat., 68:68-69, 1934), line 2, producing intersexes predominantly of the hermaphroditic type, is still segregating. The three other lines are producing practically only one type of intersexes. These lines are: (1) ♀-like intersexes; (3) intersexes of ♂-like type retaining ♀ shape of abdomen; (4) intersexes of the ♂ type. F_1's from crosses between 1 x 3, 1 x 4, and 3 x 4 lines are morphologically intermediate between lines. F_1's from 1 x 3 and 1 x 4 besides having external and internal genitalia of the ♂, also have ♀ genital plates, vagina, two spermathecae, and tubular recepticle, There are, however, minor differences in the shape of the abdomen between F_1's of 1 x 3 and 1 x 4. F_1's from 3 x 4 cross have ♂ external and internal genitalia, but the shape of the abdomen is intermediate between ♀ and ♂, although resembling more that of a ♂. F_2's from all crosses segregate in 1:2:1 ratio. Results suggest that either three allels of the gene for intersexuality are responsible for the different morphological type of intersexes, or that there are three allels of modifiers of the gene for intersexuality.

News items

Genetics Laboratory, Columbia University, New York, N.Y. - Apart from work of Schweitzer and Dunn, melanogaster is used chiefly for course work and master's problems by graduate students. Each year several students are able to carry out preliminary experiments in locating new genes and in studying interaction and dominance effects of known genes (especially eye colors and eye sizes) and material to be used for these purposes is welcome. Dunn is interested in lethals and semi-lethals like l_{hII} which have retarding effect on development.

Schweitzer has been analyzing the data on crossing-over in D. melanogaster in terms of the cytological events. He is interested in crossing-over data for other species in which whole chromosomes are covered with no more than 1/2% of undetected doubles.

Apparatus. Curt Stern - A funnel has been constructed, which facilitates the filling of food-bottles. The funnel is made of metal and has a capacity sufficient for food for about 60 bottles. It has a metal faucet which is easy to handle. The funnel is mounted on a stand, but can be detached for cleaning purposes. Below the funnel opening the stand has a hole through which extra drops of the food fall so that the bottles do not get dirty. The base of the stand has a rail which guides the bottles. This funnel can be obtained from the Will Corporation, Rochester, N.Y. A similar model has been in use for many years at the Kaiser Wilhelm Institut and has proven of great help in reducing the time and labor involved in the preparation of fly-food.

The Will Corporation has also on sale an etherizing glass following in general the design of C.B. Bridges (32) given in Amer.Nat.,66:250-273. An improvement consists of the following: Instead of a small funnel and a layer of plaster of Paris which have to be fitted and fastened into each glass individually, a one piece metal funnel and either holder is used which fits in without special fastening devices.

Inbred Bar. Charles Zeleny - Closely inbred (single pair brother and sister matings) 17° and 27° constant temperature lines are being maintained; at present (January 1934) 297 generations at 27° and 129 generations at 17°.

NOTES AND NEWS

Nomenclature

C. B. Bridges On naming allels. It is suggested that as new allels arise they be named with the numerical sequence system (L, L^2, L^4) or the more precise dating system ($w^{33\underline{1}4}$) in which the locus name is an integral part of the mutant name. It is proposed that the few allels which at present have special names (eosin, sooty) be brought into line with the above system by prefixing the locus name to the allel name (white-eosin, ebony-sooty, vestigial-Depillato). This alteration will make the names correspond to the symbols (w^e, e^s, vg^D) and will maintain the same order in lists of names as in lists of symbols. The hyphen may be used to connect the two halves of the name since the fraction bar / has supplanted the hyphen in denoting the two chromosomes of a pair. The abbreviated forms w-eosin, e-sooty and vg-Depillate could be used as equivalent to the full names.

H. J. Muller Inversions. As the number of inversions is outrunning the alphabet, and arbitrary letters newly assigned to them are both hard to remember and confusable with the symbols for gene mutations, we find it preferable to stick to the admittedly imperfect method of representing inversions by means of the "mutational" changes that accompany them, or, when these were not evident, by the arbitrary lettering originally given, with the reservation that more care should be taken to give simple designations to those hereafter arising. In designating combinations having the left part of one inverted chromosome and the right part of another, we have found it convenient simply to add the capital letters L and R, for left and right, to the symbols for the inversions, so that, for example, $y\ sc^{4L}\ sc^{8R}$ represents a crossover chromosome having the left part of the scute ^{4} chromosome, including its left point of rearrangement (breakage and reattachment) and the right part of the scute^8 chromosome, including its right point of rearrangement, together with any genes that may perforce be included, which latter it may or may not be desirable to represent, as the occasion demands. Where there is danger of confusion between the loci themselves, which are designated by the symbols, and the chromosome arrangement in question, a dot is placed next to the symbol or, preferably, below it, to show that it is the point of rearrangement together with what goes therewith, that is being referred to. Thus, in the case given, sc^{8R} happens to include the scute-8 gene itself (which could be represented in addition, when desirable), whereas sc^{8L} would not include the scute-8 gene itself, and would, where accuracy was required, be represented with a dot under it.

H. J. Muller Attached X's. For the symbolization of attached X-chromosomes, we prefer to make a line (where possible, a downwardly curved line) above the symbols of the contained genes, rather than below them, since we often use the latter mark to denote heterozygosis, and if one of the chromosomes is normal it is not always convenient to have to put the ⌿ sign underneath. Again, the line underneath is sometimes desired to represent the chromosome itself.

(Editors' remark: Underlining to designate attached X's is used in the DIS circular because this sign is available on our typewriter. To type a line over a symbol requires handshifting of the roller, which is both a cumbersome and a slow process, especially with single spacing of the lines).

H. J. Muller Rearrangements in general. No very simple system is possible for a complete and practicable representation of all possible chromosome rearrangements, but one of the least complicated, though admittedly applicable only to well-analyzed cases in which it is desired really to show the details, is illustrated by the following example of brown-Variegated ^{4}, based on data of Glass, -px⌿. st⌿:- ; :-sp⌿. .3at- Here each independent dot (period) represents a point of rearrangement. To the left of the dot is placed the designation of the chromosome (where necessary) and of the first chromosome locus know to be to the left of the break in question, and to the right of the dot the corresponding items for the latter region. In the designation of the locus, either the locus symbol, such as px, or the locus number (in this case 2,100.5) may be used, but several considerations make the letter symbol ordinarily preferable. Where it is desired to show that the genes, as represented, lie in an inverted order, an arrow pointing backwards may be used and will sometimes obviate locus designations; for typing, an arrow may be shown as a colon followed by a dash. Where it is desired to represent the locus of attachment of the spindle fibre, "at" is used, and for a free (or originally free) chromosome terminus, "tm"; the latter symbol may, however, be understood when there is a blank space to the right or left of a dash or arrow. Pieces attached to the side of the chromosome are shown in parenthesis between the loci bounding the region of their attachment (e.g., Pale is 2(px-)$^-$; 3e (.px -)ro).

H. J. Muller Deficiency. As a logical and practical extension of the system of using one basic symbol for all genes of a given original locus, with exponents in the form of letters or numbers for different mutant allels (a system which I developed while in the Drosophila laboratory at Columbia University), and with the exponent ⌿ (plus) for a normal allel (as I have done for some years at Texas, and explained in a letter of about 1931 to Stern, and as was recommended in the first "Information Service"), it will often be found convenient to use the exponent - (minus) in cases of small deficiencies, and in general wherever it is desired to designate the absence of loci that might otherwise be expected to be present. Thus, Notch 8 can be indicated as w$^-$ fa$^-$ A$^-$ or, more correctly yet, as (w$^-$A)$^-$; Notch "172b", which seems to include the originally free end ("tm"= terminus) and extends beyond echinus on the right, would, similarly, be (tm-ec)$^-$, or (-ec)$^-$.

J. T. Patterson Symbols for Translocations. We are adopting the D.I.S. symbol T for translocations, and in addition are using a subletter A (eg. T_A) to indicate that the translocation was obtained in the Austin laboratory. We would suggest the adoption of some such system as this in order to avoid confusion.

W. P. Spencer Symbols (1) I favor using the date of discovery in the symbol for the following reasons:

a. This furnishes an accurate record of the total number of recurrent mutations at a locus, whereas by methods now in vogue many apparently identical allelomorphs arising by remutation are probably not put on record.

b. It gives a simple objective symbol with a standard meaning. This avoids the confusion now arising from the diverse symbols used in different laboratories.

c. It focuses attention on the time at which mutations are occurring in different laboratories. My own experience indicates that the distribution of total natural mutations in time is not random.

Reascarch Notes

H. J. Muller Balancing of deleted X-chromosome. This may be done conveniently in cases where males having the deleted X are fertile, by crossing attached-X females containing the deleted X to scute-19 males heterozygous for Curly. Curly sons have their scute deficiency covered by the deleted X and by nothing else, and when they are crossed to ordinary females with attached X's, they immediately form a balanced stock, from which the deleted X cannot be lost except through some chromosome rearrangement.

H. J. Muller Triploids Following the construction by Miss Sarah Bedichek of the University of Texas of an improved stock of triploids, from the point of view both of recognition and of partial balancing, I have by a further modification constructed a completely balanced stock. Miss Bedichek's stock contained attached X's homozygous for yellow, and a detached X having the scute-8 inversion together with singed and apricot. Our present stock has the same kind of detached X, but its attached X's are homozygous for the delta 49 inversion and the genes white and spectacled. Diploid females with attached X's are therefore sterile because of spectacled, and those with detached X's are sterile because of singed. Crossovers appear very rarely. It is well, however, to add extra males. A useful mark which I have noted for the recognization of triploids consists in the reduction of the "ventral" bristles (between the bases of the first and second pairs of legs); the great majority of triploids have at least one of these absent, while in diploids (normal for scute) they are practically always present.

H. J. Muller Extension of third chromosome.

A translocation recently studied, known by the name of the mutant character "scute^{J4}", involved in it, has shown that the third chromosome extends at least several units (4±) to the left of roughoid, since this much crossing-over occurs between the attached piece of the X-chromosome (containing y and scute^{J4}) and the locus of roughoid, while the locus of hairy remains with that of roughoid during this crossing over. Stock is available containing scute^{J4}, roughoid and hairy in the same chromosome, and having yellow in all the X-chromosomes (attached X's of females and detached X of male) so that yellow may here be used as if it were a marker for the left end of chromosome 3.

N. Timoféeff-Ressovsky Temperature-experiments with Drosophila melanogaster.

The finding of Muller and Altenburg (1919) and of Muller (1928) that the spontaneous rate of mutation follows the Vant'Hoff's rule was confirmed: the rate of sex-linked mutations is in 25°C about 3 times as high as in 15°C, the difference being statistically significant (Diff/m diff=5,8).

Temperature-shocks (15-24 hours in 35° - 37 C) were applied at different developmental stages in males (adult, 3-6 days old larvae), using the "ClB"-method and the "attached X"-method for detection of sex-linked lethals and visibles; the results of these experiments (started in October 1927) showed only a slight, if any, increase of the rate of mutation: a.11:2993 as compared with 4:1362 lethals in the controls ("ClB" experiments) and b.13:65997 sex-linked visibles as compared with 6:58453 in the controls ("attached X"-experiments).

N. Timoféeff-Ressovsky A comparison of the mutation-inducing effects of X-rays and Gamma-rays.
7

The dosages (in r-units) of Gamma-rays of Ra published in the last papers of Hanson and Heys suggested (according to the mutation-rates induced by these dosages) that Gamma-rays are about 3 times less effective than X-rays in inducing mutations (0,78% sex-linked lethals per 1000r Ra in Hanson's experiments, as compared with about 2,5% per 1000r X-rays). Since there are no theoretical means to admit the existance of such a pronounced difference, and the measurement of Ra-rays in r-units offers some difficulties, and exact comparison of the mutation-inducing effects of equivalent dosages of Gamma- and X-rays was made (using the "ClB"-methos and raying adult males). Different dosages were applied and the mean rates of sex-linked mutations per 1000r were; 2,48% mutations per 1000r Gamma-rays of Ra and 2,43% mutations per 1000r X-rays, the difference being insignificant.

Technical Notes

M. Demerec Flexible chromosome maps

For preparing chromosome maps on which single items can readily be changed and order or position of different items shifted, we are using "Kardex wall panels for tubes", with tubes 6 inches long and 3/16 inches wide. A 20 inch panel holds 90 tubes which is enough for the map of melanogaster X-chromosome. Since the space for each item is 6 inches long it gives room enough for: locus, symbol, full name and RK. By inserting differently colored transparent markers in tubes we designate loci which are carried in our stocks, which have been lost, etc. The material may be ordered from Remington Rand Inc. 205 East 42 Street, New York. The price is $3.00 per panel.

B. H. Glass Control of mold and mites.

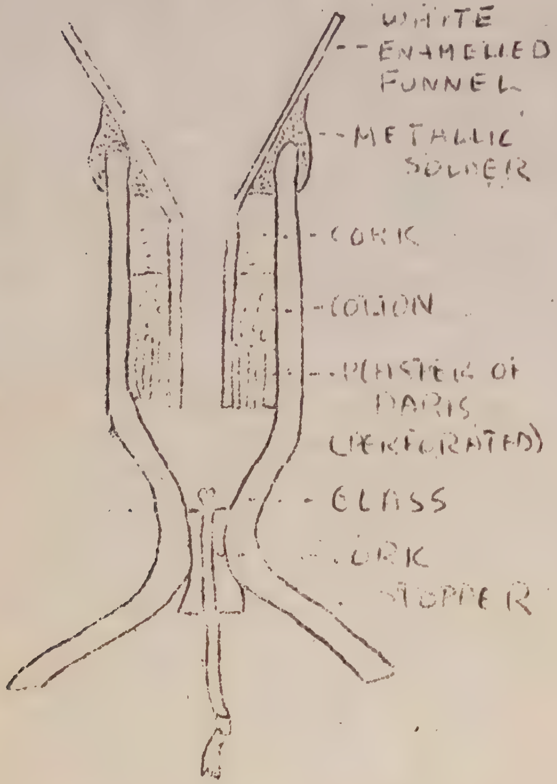
In combatting a severe infection of mold in Drosophila cultures, it has been found helpful to hold individuals for two or three days in vials of food containing 0.2% formaldehyde, added when the food is prepared. Flies can live for several weeks in such vials, inoculated in the usual manner with yeast; but larvae do not develop after hatching; and mold growth is inhibited. These vials are also very useful for holding individuals to be mated at some future time. Before transferring to fresh food, the flies are given a bath in a watch-glass of 70% alcohol for 2 to 3 minutes; then dried on filter-paper. Flies will stand a considerable immersion in alcohol with no permanent ill effects. They may be handled readily with brush and forceps. The alcohol bath treatment is also effective in freeing flies from mites. Larvae are especially easily cleaned in this way, the mites coming off at once; whereupon the larvae may be touched on filter-paper, and transferred at once to the food, using a long-handled needle, to which they gently adhere.

M. Demerec Control of mites.

As a preventive measure against the spread of mites we are keeping stock cultures (and also all other culture bottles which are used during a long period) standing in a weak solution of creosote. For this purpose shallow (2 inches or 5 cm high) galvanized iron trays are used. These are made to order to fit our shelves (usually 12 x 36 x 2 inches). In case any of the cultures is infected with mites creosote solution prevents their spread to adjacent cultures and keeps the infection under control. Some of our trays have been in use for over five years without any sign of wear. The initial cost for trays, therefore, is spread over a long period. Mites can also be controlled effectively by avoiding accumulation of old culture bottles and by wiping frequently, shelves and tables, with carbon tetrachloride.

Technical Notes

M. Demerec Etherising bottle. The following drawing represents an actual size cross section of the etherising bottle used in our laboratory. The design is a slight modification of an early Bridge's design. Ether is poured in thru the bottom hole. A few drops suffice for one hour's work. Specifications: Funnel: White enamaled 1/8 quart improved funnel manufactured by the Vollrath Co., Sheboygan, Wis., obtainable in hardware stores or could be ordered thru a hardware store. Top cut off to fit the culture bottle and bottom cut off to fit the glass vessel. Cost 30-40 cents. Glass vessel: Can be made by any glass-blower. We order it from Eck & Krebs, 131 West, 24 Street, New York, at 50 cents a piece.



H. J. Muller Etherising bottle. The type which I have found most practical all round (and at the same time the easiest to construct, repair and clean) consists of a glass containing-vessel (whose shape may be chosen according to the convenience of the operator), into the neck of which is firmly fitted, preferably through the mediation of a hollowed-out cork, a funnel, preferably of metal, with an upper end wide enough to fit against the mouth of the widest-mouthed culture vessel used. To the narrow end of the funnel, below, a large gelatine capsule of the same diameter, and containing numerous fine needle-holes, is glued on. In the space between the bottom of the suspended capsule and the bottom of the containing vessel is packed a mass of dense cotton, (which must not touch the capsule). Flies go in and out through the same opening, without manipulation of any stoppers being necessary and are quickly etherised by the diffusing ether. The ether used need usually be added to the bottle but once a day, as a minimum is lost (an advantage both economically and physiologically). If desired, glass can of course be substituted for the gelatine, but the latter has considerable durability and the ether can be poured in directly through the gelatine sieve. The latter is later wiped quite dry with a paint brush.

Technical Notes

H. J. Muller Stock lists. There can be no universally satisfactory order for listing or arranging stocks. We feel that the arrangement should usually be a functional one, it being more useful for the purpose of some laboratories to put together all stocks with a certain kind of inversion, regardless of their alphabetical place, and more useful for others to group them according, for example, to the alphabetical location of the leftmost gene mutation or chromosome abnormality contained, but where, for instance, there are many inversions which change the order of the genes, it is confusing to let the arrangement of stocks be altered by the latter. A system we have found useful has been to separate stocks with single mutations from those with more, and these again from all cases of chromosome abnormality, but all needs can be filled fairly well at the expense of a longer list, by having various simultaneous methods of classification in one general cross-index. We give an illustration of this, at least so far as the sex chromosomes are concerned (the other group being too small to require such treatment), in the stock list we are submitting. (This list is, however, admittedly very incomplete for chromosome abnormalities, since we notice that in general these are not being listed by other laboratories.) As an aid to flexibility in listing, I have found the "Cardex Visible Index" useful, such as is used in posting names of registrants at a convention, but if an ordinary list is made in the form of a single, instead of double, column of stocks, it can easily be cut and pasted together behind, for the insertion and deletion of stocks.

H. J. Muller Concerning acknowledgements. It is difficult to understand the rationale of the announcement on the cover of the circular that "material presented in this circular must not be used in publications without the specific permission of the author", as the obvious purpose of the circular is to furnish useful information for Drosophila workers for researches that they will publish. Every contributor must take it for granted that the data or suggestions which he allows to be distributed in the circular may be put to use. Surely the latter are not merely for the inner edification of the readers. Therefore it should be as unnecessary as it is impossibly cumbersome for every user of the material in the circular to have to write to every contributor involved every time he intends putting a bit of the material to use. To be sure, the injunction on the cover implies that the contributor's consent need be obtained only when the information is used "in publications". If, however, it should be obligatory for the user of the communicated material to obtain the consent of the author of the latter in mentioning it (presumably with acknowledgements) in a publication, it must surely be even more obligatory for the user not to secretly take the information given and use it without acknowledgements, in the obtaining of further data or conclusions which he then presents in a publication, for in the latter case the prerogative due the original contribution would be even more infringed upon. Clearly then such information used in work that reaches publication requires acknowledgement, but since, by the system proposed on the cover, all acknowledgements in publications require the consent of the first author, it would then become necessary for the consent of the author to be obtained for each occasion in which his material is used.

It would appear then that the actual ethics of the matter should be almost the opposite of that which seems to have been proposed in the announcement. That is, it should be assumed, firstly, that the information or suggestions in the circular should be used as freely as possible, without obtaining the consent of the contributor, as the latter gave them freely. It should be assumed, secondly, that exactly because the material was not presented in copyrighted form in an official publication, wherein the general scientific public could plainly see its source, it is incumbent upon him who uses it to acknowledge his use of it and the source from whom he obtained it when he publishes material of his own, in the development of which the use of the first mentioned material was involved. This matter, in other words, must be upon the same footing as in the case of material (data, conclusions or suggestions) passed on by word of mouth or by letter, where of course it is the most obvious and elementary principle of ethics that acknowledgement of the source must be made if the scientific material is put to published use. Of course it is not to be expected that acknowledgements need be made in respect to the use of minutiae of technique etc. that the author would not think worth mentioning anyway, even in case he had originated them himself, not are acknowledgements to be expected where the material made use of and its source have already become generally known in some way, not where the points involved would be generally recognized as being quite obvious. Where on the other hand a method or result that would generally not be regarded as obvious, has been originated independently by a given worker, but parallels similar work or suggestions that have later become known to him through public or private, direct or indirect communications of another investigator, it is obvious that the former, in publishing his work, need not acknowledge assistance from the latter, but is nevertheless in duty bound to mention the fact that his method, conclusion or suggestion, though independent, is the same as that which he knows the other worker has also arrived at. Thirdly, far from the consent of the original author being required for acknowledgements, his consent should rather be required in case it is desired not to make mention of a communication of his which has played a role in work that the other author is publishing, and this requirement has much more pertinence in a case like that under consideration, where the communication was not made in an official publication, than where it was so made, since in the latter case the original author has, after all, a certain amount of protection in the words themselves. Only on such an understanding can contributors to the circular be expected to continue to furnish contributions, just as they would only (by word of mouth or letter), so long as such a standard of conduct was observed. Experience has shown that it is unfortunately only too true that such overt understandings must be reached beforehand, even in the case of scientists, in order that real cooperation may be possible.

H. J. Muller Concerning material to be published. It is not always possible to know at the time of sending in a contribution just whether or not the material will be published, or, if published, when, but it would seem wise to send it in to the circular anyway (supposing the policy on acknowledgements above outlined is accepted), provided the information or suggestions sent in might be of considerable use to the readers between the time of appearance of the circular and of the official publication. This would apply especially in cases where actual publication is only doubtful, or might be long delayed. In other words, it would seem wise not to limit the material in the circular in the manner stated, to material which is "not ordinarily suitable for publication", or to material, the publication of which is not expected at all. Certainly a good deal of the material in the last circular would be worth eventual publication.

M. Demerec Drosophila stock center at Cold Spring Harbor.

Under cooperation of the Carnegie Institution of Washington, the Rockefeller Foundation and the A merican Society of Naturalists a stock keeping center has been established at Cold Spring Harbor for supplying stocks for research purposes to laboratories located in the eastern section of the United States and in Europe, and for serving as a connecting link for shipments to European laboratories. This center is cooperating with the Pasadena Drosophila laboratory in keeping valuable stocks at both places and thus insure against their loss.

At present a complete set of melanogaster loci representing X-chromosome, RK1 to RK3 mutants of autosomes and a set of complete stocks representing most useful combinations are available. Also an almost complex set of virilis stocks is being kept here.

The Carnegie Institution of Washington made an arrangement with the U. S. Department of Agriculture, Bureau of Entomology by which the Carnegie Institution and its cooperating agencies are authorized to import and move interstate, living material of various forms of Drosophila under stated conditions. This arrangement covers shipments between the Cold Spring Harbor and the Pasadena stock centers and the laboratories cooperating in the DIS project.

NOTES AND NEWS

Nomenclature

D. R. Charles Translocations In glancing again over Muller's "Rearrangements" note in DIS:2, I can not help feeling that his symbols are a bit too compact, at least for people who are not working a good deal of the time with translocations. But some symbology is certainly needed and Kaliss and I are using a basis for nomenclature which is pretty well shown by the symbol for T,3-4c: 3L•cu; 3R cu•4. (a) where more than one chromosome is involved in the rearrangement, the resulting elements are arranged in order of the spindle fiber to which they are attached - here 3L•cu has the third sf, 3R cu•4, the fourth; (b) as with Muller, the dot indicates a rearrangement point and where possible, in each part of the broken chromosome is shown the known locus (in that part) which is nearest the rearrangement point; (c) no cumbersome and confusing arrows are needed: had, for instance the right end of the 3 been attached to 4 in T3,4 it would have been written •cu 3R•4. Here are examples: Dobzhansky's (1932) Genetics 17: 369-92 translocation would be: 2R vg•B 1R; 2L cu•f 1L; Van Atta's (1932 Genetics 17:637-59) Dilute-1 would be: •pr 2R•2L b, which says "this chromosome starts to the left of pr, goes to the right end of 2, continues thru the left end of 2 to b." ClB would be: 1L ec•sy bi•fu 1R.

H. J. Muller Nomenclature of allels. It is evident that the nomenclature proposed for allels, involving the date of discovery, too cumbersome ordinarily to be used in formulae, and that in practice, after the first definition, an abbreviation would be employed so that the first abbreviation would have purposes only of reference, This being the case, the abbreviational character of the first symbol becomes of minor importance. Since there seems no interest in itself attaching to the exact date of discovery of a mutation, and even the record of that may, for this reason, not have been kept, different investigators having different methods of work and laying emphasis on different aspects of their investigations, it may be questioned whether it would not be better to let each investigator, or group of investigators, list their own mutations: for example, numbering them as they wish, and giving them the name of the investigator or group. The latter name can usually be abbreviated to one or two letters. In this way it would be more easily evident to whom a person interested in a given allelomorph should turn, if he wishes more information concerning it, and the abbreviation might well be kept within reasonable limits of size in most cases. In proposing this, however, we do not wish to give the impression that we consider it important to know who the original finder of a given mutatuion was. The finding may have been made as a purely routine matter or an assigned problem, by a technical assistant or a student. The planning of the tests of the mutation (localization, etc.) and the interpretation of the results may have been the work of a second person, and the actual manipulations may have been carried out by a third, or even by several in cooperation if the case were complicated, while still another might have made up the final stock. In such cases, names have little meaning, except in so far as it may at some time be useful to be able to consult the director or directors of such work.

Research Notes

L. C. Dunn Retardation effect of minutes

Retardation effect associated with Mw, M33j25, Mh, Mz, and M12 confined to egg-larval stage. M6 and M12 tested also for retardation in egg stage alone. These do not retard growth of embryos. Order of retardation effect at 25 degrees from most extreme (larval period three days longer than normal) to least extreme (larval period three days longer than normal) = Mw, M33j25, Mh = Mz, M12. None of these retard development of pupae. Mw and M33j25 also act as pronounced minus modifiers of Lx and L4 heterozygotes; M12 as slight minus modifier.

S. Gershenson Mechanism of chromosome conjugation.

The following conclusions were obtained in a preliminary study concerning the mechanism of chromosome conjugation in flies heterozygous for an X-chromosome containing a long inversion (In y^4-w^a, y-sc^4 8 sc^8-w^a studied).(1) Double crossing-over is approximately normal; (2) Primary exceptions among females are rare, but nevertheless somewhat more often than normally; (3) Primary exceptions among males are frequent (+1.5-2%); nearly all of them probably result through the loss of the maternal X due to crossing-over; (4) Heterozygous females produce a large percentage of perishing eggs, indicating a high (perhaps nearly normal) percentage of single crossing-over; (5) Secondary exceptions are significantly more frequent than usually (+9-13%), indicating a marked decrease of synaptic affinity between the non-inverted and inverted X's.

H. Kikkawa A dominant eye color mutant in D.virilis.

It is assumed that dominant eye color mutant found in D.melanogaster are usually correlated with chromosome rearrangements except Henna described by Van Atta (Muller 1930, Van Atta 1932, Glass 1933, Schultz and Dobshansky 1934). But it seems that no attention has been paid to the dominant eye color mutant Garnet (3-108.5, homo viable) discovered formerly in D.virilis (Metz, Moses, Mason 1923, p.39). To test whether or not any chromosomal aberration is associated with this gene, females of the constitution sv/cn G and sv/cn were mated to sv (short-veins 3-90.0) cn (cinnabar 3-107.0) males respectively. But the result was negative (the difference of recombination percentage for sv-cn region in the two experiments is only 1.6 times the probable error). The former experiment: (0) 595(sv) + 601(cn G), (1) 92+ 60, (2) 7+16, Total 1371; R_1=11.2%, R_2=1.6%. The latter experiment: (0) 461(sv) + 414(cn), (1) 56+71, Total 1002; R_1=12.7%.

D.E. Lancefield Linkage in pseudo-obscura.

Some linkage crosses involving compressed^2 and short D.pseudo-obscura Race A are suggestive that more than fifty per cent of recombination may be obtained between these two loci. Further tests are being made to see if this result can be confirmed.

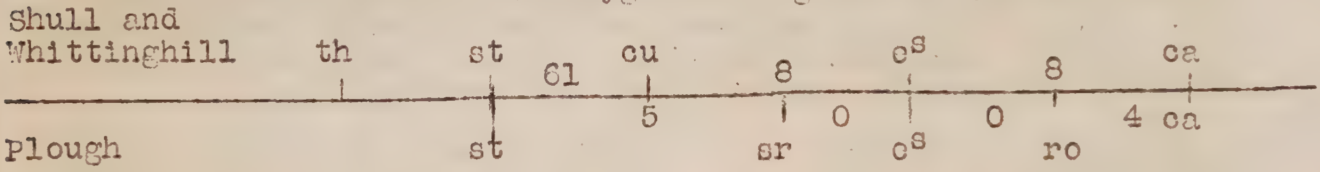
H.J. Muller Balancing chromosome I with scute^{S1}

A rather extreme scute allel, found by Sinitskaya in March 1934, analyzed genetically by Muller and cytologically by Prokofyeva, includes a long inversion having its left break close to the right of scute and right break in inert region, and also a small inversion, somewhat smaller than delta 49, and having the positions of both its breaks included within the positions of the two breaks of the latter. No crossovers were found between sc^{S1} and normal chromosomes in a count of 700. Homozygous sc^{S1} females are sterile and have rather low viability; males are fertile and with fair viability. This therefore provides a very convenient balancing chromosome for many sex-linked genes, etc.

H.H. Plough Crossing over in male D.melanogaster following heat.

At the meeting of the Genetics Society at Woods Hole in August 1934, I reported finding 6 cases of crossing over in chromosome 3 following exposure of 5 day old larvae to 36.5° for 18 hours. Although it was unknown to me at the time, Shull and Whittinghill had reported in July 1934 (Science 80:103-4) 77 cases in the same chromosome, of which 32 were all of the same type and derived from a single male. While neither investigation is complete it is of interest to summarize these two series of tests up to this point.

Crossovers in Males heterozygous for genes in Chromosome 3.



My results do not show correspondence with those of Shull and Whittinghill in the ratio of crossovers in the st-cu-sr region and this fact suggests perhaps that their irregular group of 32 may need to be reinterpreted. In any case the distribution of the whole series indicates that crossing over in the male may take place in any region of chromosome 3 following exposure to heat.

N.W. Timofeef-Ressovsky Experiments on intraspecific evolution in Drosophila.

Ecological and physiological experiments with different Drosophila-species are of importance in connection with the genetic analysis of evolutionary and zoogeographical problems. A good method of testing "adaptations" is the determination of the "relative viability" of different mutants and biotypes under different environmental conditions (see my paper in Z.Ind.Abst.Vererb.,66:319-344,1933). Some difficulties arise when geographically different normal populations of a species should be compared in respect to their "relative viabilities", since they can not be distinguished phaenotypically, and hence the methods of backcrosses or of counting flies in overcrowded bottles containing equal numbers of eggs of the two types to be compared can not be used. In these cases another simple method can be applied. Different populations of one species can be compared, in respect to their "relative viabilities" under certain conditions, with a pure-bred, standard stock of another species, using the method of overcrowded bottles with equal numbers of eggs of the two types (see my paper in Arch.f.Naturgesch., 2: 285-290,1933). This method was applied in studying the "relative viabilities" of geographically different populations of Drosophila melangaster and Drosophila funebris under different environmental conditions (food, moisture, temperature), using a standard inbred melanogaster-stock for testing different funebris-populations, and a standard inbred funebris-stock for testing different melangoaster-populations. In small culture-vials with food (yielding normally about 100-120 flies) were put 150 (or 200) eggs of the standard stocks of one of the species and the same number of eggs of the population of the other species to be tested; the number of hatching flies of each species were counted, and the tests were repeated until large enough numbers of flies were obtained. The different populations of one species could so be compared inter se, using as a scale their differences from the same standard stock of the other species. These experiments are not yet completed; but the results already obtained show that many of the geographically different wild population, although morphologically indistinguishable, can show remarkable hereditary differences in their physiological properties, a part of these differences being clearly of the type of ecological adaptations. At the same time, experiments of Muller and of myself showed that mutations producing only slight deviations from the "normal relative viability" are produced by x-rays at a rate about twice as high as that of the lethals (Muller's paper read at the 4.Intern.Radiol. Congress and my paper in Strahlentherapie,v.51). Such "slight physiological mutations" are probably also the most common type of spontaneous mutation. These mutations are probably used by natural selection in order to differentiate the species into biotypes and races, adapted to different geographical environments.

Technical Notes

Margaret E. Hoover
Transportation of
Drosophila cultures

For mailing Drosophila, we have been using 7 x 2cm. shell vials. The vials contain a small amount of the usual corn-meal-agar prepared food, innoculated with yeast, and a strip of paper is inserted to prevent the food from running onto the sides of the vial. From one to seven vials will easily fit into corrugated paper boxes (8 1/2 x 6 3/4 x 6 3/4cm) If the vials are wrapped in paper and tightly packed on all sides by cotton, there is no danger of breakage. We have found this to be a very satisfactory method for transporting stocks. Both Drosophila melanogaster and virilis cultures have been satisfactorily shipped as far as Japan. The mailing costs are low. The packages may be sent third class in the United States and as small packets or samples to foreign countries. A full package will usually not weigh in excess of six ounces.

J.C. Li Isolation of
larvae

In the Yenching Laboratory we have devel-oped a technic by which not only eggs but also larvae of D.melanogaster can be isolated within one hour of their hatching. It is essentially the same technic developed by Li (see Li '27 appendix p.55-57). The eggs are allowed to hatch and the newly hatched larvae can then be removed from the food with a tiny dissecting knife. By means of this technique it is possible to analyze more exactly the critical period when a certain gene operates during the larval period. It is now also possible to study the embryology of Drosophila more exactly under controlled conditions.

H. J. Muller Labelling of stock cultures.

In place of the usual practice of Drosophila laboratories of pasting a label on each stock culture and writing the name of the stock anew at each transfer, I have for many years found it much quicker and less subject to error, if the designation of the stock is written once for all in ink or India ink on both sides of a cardboard tag which is affixed thru its string to a rubber band that passes around the neck of the culture vessel. This tag is transferred to the new vessel when the flies are transferred, and it is best to have a separate tag for each culture vessel.

H. J. Muller Fly morgue.

In place of the usual method of having a jar of alcohol or other volatile fluid into which the flies to be discarded are dropped thru a narrow slit, it is much more convenient to have a broad dish containing a non-volatile oil. The used oil from automobiles affords a conveniently obtained medium. The opening may be protected by a wide-mesh wire grating. The flies do not have to be brushed off in any exact manner, but may be merely jarred off by knocking the porcelain plate against the screen with one motion of one hand. Renewel is seldom necessary and there are no disturbing odors. This method was used independently in Texas and in the USSR.

H. J. Muller Seeding with yeast.

In place of the usual method of allowing drops of yeast to fall into the bottles from a pipette or sprinkling crumbs of yeast, it saves time and ensures more even distribution if one makes up a very thin suspension of the yeast in water, and then sprays this through a simple atomiser, such as is used for spraying fixative on charcoal drawings. In this way a great number of cultures may be seeded at once en masse.

H. J. Muller Supplying vials with paper.

When numerous small vials have to be handled it is time-consuming to prepare and insert paper for each one, although the presence of paper is helpful. For this purpose it is convenient to use white paper confetti, which can be purchased already prepared in considerable quantities. This is sifted between the fingers into the cultures en masse, as they stand still uncovered after having been seeded with yeast.

C. A. Offmann and I. K. Schmidt
Culture media for Drosophila.

With the development of the Drosophila technique, not only a certain amount of sterilization of the culture medium during its preparation became necessary, but also an adaptation of it at different requirements. Productivity and duration of the media are the two main factors to be considered for our purpose, and they are to a certain degree in inverse relationship. By productivity we mean the quantity of flies produced in a given time. By means of overcrowding a certain food can yield a higher number of flies which are small in size, but this higher yield will be usually cancelled by a serious loss in the speed of development (in strongly overcrowded bottles in fact the cycle has proved to be as much as twice the usual length). Three functional types of media may be distinguished: 1) for the maintenance of parent flies, 2) for the maintenance of lines of stock cultures, 3) for the attainment of high productivity. 1)This type has proven to be extremely useful for the current work where we have to keep alive the flies from the moment we obtain them until the moment of their use. In this case offspring are not desired. Flies have been kept on such a medium for over a month (some over two months) at room temperature, without a transfer. The same vial or bottle can be used over again until the surface dries out, and etherized flies will not stick to its surface:- Water 90cc., agar 2gr., syrup 7gr., Nipagin .15gr. 2)Suitable media serving this purpose, such as the banana agar and the cornmeal syrup media, are already in use in all Drosophila laboratories and will not be described here. 3)The main characteristics of this type are: production of large quantity of flies, short cycle of development, and low selective level (preservation of individuals of low viability).

The addition of killed yeast in large quantities to the ordinary food formulae was introduced a few years ago by Muller (in 1928), giving surprisingly good results. These media had, however, the inconvenience of requiring a constant supply of fresh ingredients. Dry yeast was used in place of fresh yeast by Winchester and by Gershenson. The authors have recently experimented with a systematic series of modifications of the Russian food mixture with the addition of dry or fresh yeast. Fifty different modifications have been tried, approximately twenty vials being employed for each trial and counts of the offspring made. Each ingredient was tested in different concentrations. As a result the following formulae have been found the best for obtaining high productivity. (A.with dry yeast) - Water 80cc., Agar 1.5gr., Dry yeast 1.5gr., Raisins 4 gr., Syrup 5 gr., cornmeal 5gr., Nipagin .15gr. The agar is dissolved by bringing the water slowly to the boiling point, dry yeast (that has been disintegrated in a small part of water) is added and the mass is kept boiling for another ten minutes, so as to make sure that all the yeast cells are killed. Then the mashed raisins, syrup and cornmeal are added with continuous stirring, and the food will be ready for distribution. The addition to the liquid mass of "Nipagin T" Nachmittelfabrik Julius Penner A.G. Berlin Schoeneberg as found in Dr. Nachtsheim's laboratory, is important for cultures which contain few larvae or develop slowly.

The layer of food should be somewhat deeper than 1/2 inch and its surface seeded with pure live yeast (fresh or dried). Adding paper and making the suface appetizing with fruit juice did not increase the yield in our case. 200 flies per vial and 1000 per half pint bottle should be considered a good average. This means that a vial can be employed where formerly a bottle was required, and a bottle can take the place of a group of bottles. Not only the number, but the size of flies is considerably increased. When fresh yeast is easily available it can be employed advantageously by substituting 15 grams fresh yeast for 1.5 grams of dry yeast in our formula.

The preceding formulae enable us to prepare food of each of the three types by the use of ingredients which will not spoil. A laboratory can thus provide itself with a year's supply at once, avoiding further trouble in this connection.

We desire to call special attention to the convenience offered by the new type of medium here described: the "syrup-agar" for the preservation of the P flies, for the great elasticity it introduces in current laboratory work.

E.E. Shipman Bottle for Drosophila culture.

Due to the high cost of transportation of the bottles designed by Bridges and manufactured by the Owens-Illinois Pacific Coast Company at San Francisco, it was necessary to find a substitute bottle manufactured nearer home. The writer has found a Urine Specimen Bottle, No.820, manufactured by the Glasco Products Company, Chicago, Illinois quite satisfactory. The bottle is made of the same type of glass as milk bottles, has straight sloping sides, the inside top diameter is about 1/4 inch less than the inside bottom diameter, and has a milk bottle type opening so that paper caps may be used if desired. The writer handled three gross of them this summer with an average of about 35 offspring per bottle and had only four cases where the food cake shook completely loose, daily removals were made so that the danger of loose food cakes was much greater than in routine stock work.

Reasearch Notes

Coyne, J. Crossing-over within a closely linked group of genes near locus 40.4 of Chromosome 3.

Preliminary tests for the location of M 33j (Demerec, x-ray induced) showed it to be between hairy(26.5) and thread (42.2) in the third chromosome. With the intention of using Dichaete as a marker M 33j was crossed with Ly/D $. The combination /L Ly/M33j proved lethal, since there were no Ly/M33j survivors in a total of 2416 flies. This led to testing M 33j as a /t possible deficiency for all available genes in the 40.4 group around D. Those tested included Glued, Minute-h, Lyra and Dichaete itself. The combination with Lyra was the only one proved to be lethal. The Mh/M33j combination is also probably lethal, but further tests must be made. Mh is not lethal with either Ly, D, or Gl. This evidence made it seem more unlikely that all five of these genes are uni-local. Crossing-over tests for all viable combinations of the five genes gave the following results: (Gl/D was not tested since Plough's evidence (Gen.20: 42-60) shows that there were no crossing-over between these two genes in 5000 test flies).

Mating	No. of crossovers	Total No. of flies
D/se Mh x seple	D-M h=0	484
Ly/se Mh x seple	Ly-Mh=2	1335
Ly/se Gl x seple	Ly-Gl=10(.61%)	1933
Gl/se Mh x seple	Gl-Mh=24(1.4%)	1046
se D/M33j x seple	D-M33j=0	298
se Gl/M33j x seple	Gl-M33j=0	772
Ly/D x M33j/Payne	Ly-D=0	2416

Since the 10 crossover flies between Gl and Ly were of the two phenotypes se and Ly Gl, it is probable that Ly lies to the left of Gl, unless these 10 were all double crossovers, which is unlikely. Since there is no crossing-over between Ly and D or between D and Gl, it is likely that D lies between the two. As the combination M33j/Ly is lethal, M33j may be a small section deficiency to the left of and including the Ly locus. Similarly, Mh may be a deficiency just to the left of and including part of the M33j deficiency. This possibility is further confirmed by the large crossover percentage of 1.4 betwen Gl and Mh, which places them at the two extremes of the region under observation. From these experiments the probable order from left to right of these five mutatnts would be Mh, M33j, Ly, D, Gl, covering a total map distance of 1.4 units. Further tests will be made to get larger numbers for D/M33j and D/Mh crossovers, and to prove definitely the inviability of Mh/M33j. My, an allelomorph of Mh recently received, will also be investigated.

Dunn, L. C. Effects of minutes on developmental rate and on eye size of mutants.

Supplementing report in DIS-3, Ml has retarding effect on larvae, similar to Mw. Mw, Ml, M33j, Mz and Ml^2 act as minus modifiers of $L/+$ and $L^4/+$ in order given, also as minus modifiers of L/L. Mw, Ml and Mz are lethal in pupal stage when combined with $L^2/+$. Mw, M33j and Ml^2 are minus modifiers of $B/+$ and B/B. Mw, Ml, M33j and Ml^2 are lethal or of very low viability with $cy^D/+$; Mw, Ml and Ml^2 are of very low viability in combination with $Dfd/+$.

L and L^4 prove to be very sensitive to changes in larval growth rate brought about by culture conditions (temperature, crowding). Retardation of early growth results in increase in size of Lobe eye; effect especially marked when a Minute is also present, often resulting in eyes larger than wild-type.

Bar and Lobe have cumulative effects in reducing eye size; Bar Lobe flies often show Bar in one eye, Lobe other.

Neither L or L^2 has any marked effect on developmental rate.

Gottschewski, G. Eine deficiency ohne einen genetisch bzw. cytologisch nachweisbaren Ausfall eines Chromosomenstuckes.

Nach Hitzeexposition eines ss/ss ♀ (18-24 Std. in 35-36°) wurde von Goldschmidt mehrere Male eine Mutation gefunden, die vor allem durch Kerben an den Flügelspitzen gekennzeichnet waren (phanotypisch = cut-Beaded-Kombination). Die mutation wurde von mir $Notch^G$ genannt. $N^G/+$ ♀ x fa ♂ (34j23): F_1 ♀ N^G fa 56 ♀ + 61; ♂ + 71. $N^G/+$ ♀ x spl ♂ (35f17): F_1 ♀ N^G spl 41; ♀ + 53; ♂ + 59. $N^G/+$ ♀ x ec ♂ (34j23): F_1 ♀ N^G ec 156; ♀ + 163; ♂ + 157. $N^G/+$ ♀ x ec ♂ (35e18) F_1 ♀ N^G 9; ♀ + 7; ♂ + 9. Demnach liegt eine Df für fa und spl vor. Ob die Df immer den Locus ec einschliesst, ist noch zu entscheiden. Ein Verlust einer Chromosomenstrecke erscheint nach den genetischen Befunden unwahrscheinlich, da der Faktorenaustausch zwischen Genen, die Df einschliessen, nicht kleiner, sondern grosser ist (Vgl. Linkage data). Der cytologische Befund: Carmin-Eisessig-Quetschpräparate zeigt eindeutig, dass die Banden in der fa-region unverändert sind. In keinem Praparat hat sich eine Abänderung von der für Deletionen bzw. Chromosomen-deficiencies typischen Struktur nachweisen lassen.

Hoover, Margaret E. Salivary limits of delta-49 inversion.

Delta-49 (dl-49) inversion which has been found and analyzed at the Austin Laboratory is a useful x-chromosome balancer since it is not lethal when homozygous and it prevents entirely crossing over from cv to g and reduces it greatly in other regions of the chromosome. A study of good salivary chromosome preparations seems to indicate that cytologically dl-49 extends from 4D2 to and including 11F3. A well spread figure was found in material heterozygous for dl-49 in which the x was split from the left end of the inversion through to the chromocenter, one half of the split being the normal and the other, the inverted half. In such a figure the bands could be carefully followed along the length of the chromosome to the end of the inversion where the matching bands were found in corresponding positions. This figure was also checked by C. B. Bridges. Although the similarity in size and shape of 4D1 and 12A1 make it possible that this interpretation is incorrect, evidence seems to indicate that 4D1 and 12A1 are the outside limits of dl-49.

Karp, M. L. The distribution of mutant genes affecting the number of sternital bristles in chromosome 3 of D. melanogaster. In chromosome 3 of D. melanogaster the presence of at least six mutant genes affecting the number of sternital bristles, independently of the possible effect of the gene markers, has been shown. These genes possess a considerable power of action, approximately 5 to 15 per cent of the manifestation of the character. Being opposite in tendency and alternately located, they are more or less balanced, not only along the whole length of the chromosome, but within its small regions as well. In the chromosome, causing the reduction of 5-6 bristles on 2 sternites of the abdomen, were detected genes which determine conjointly the reduction of 18-21 bristles on the same 2 sternites, and on the other hand there were found genes which together intensify the character by 12-20 bristles. Hence the genic balance of the chromosome examined offers the possibility of a considerable change as to the extent of the manifestation of the character.

Kaufmann, B. P. Drosophila ananassae (D. caribbea) In the autumn of 1933, D. caribbea was collected in the vicinity of Tuscaloosa, Alabama, which is considerably north of the range of distribution of the species as indicated by Sturtevant. Male flies of this stock have a J-shaped Y-chromosome, whereas the stock used by Metz (1916) had a rod-shaped Y. Recently a Nipponese stock, secured through Dr. W. P. Spencer, has been examined. This also has a J-shaped Y. Additional material, especially from America, is desired for further study.

Kerkis, J. Sex-Linked vestigial like mutant in Drosophila simulans On May 28, 1935 a single male was found in a normal mass culture of D. simulans which was like a vestigial of D. melanogaster. This male was crossed with normal simulans ♀. The F_1 was normal. Flies from F_1 were inbred and in F_2 there were 269 normal ♀♀, 105 normal ♂♂, and 81 vestigial ♂♂. There were no vestigial ♀♀. Males from F_2 were crossed to their sisters and in F_3 homozygous flies were produced from which a stock has been propagated. One of the ♂♂ was mated to a yellow white attached ♀♀ of D. simulans and gave in F_2 308 vestigial

♂♂ and 254 yellow white ♀♀, 2 normal ♀♀ and 3 yellow white ♂♂. The latter two classes were produced by separation of the attached X's of the yellow white ♀♀.

The data on the location of the new mutation show that it is located in the right end of the X-chromosome.

Kikkawa, H. Systematics of Drosophila.

While examining the salivary chromosomes of various species of Drosophila I realized that there are (at least) two different groups with respect to the ratio of the total length of autosomes to length of X-chromosome, viz., the one giving the ratio of about 4:1 and the other, about 1.8:1. D. melanogaster, virilis, funebris, ananassae, repleta, etc. belong to the former group, while D. pseudoobscura, affinis, miranda, etc. belong to the latter. Morphologically, there is also a distinct difference between the two groups in the shape of testis. These characteristics may be worthy of dividing the genus Drosophila into two subgenera. My inference proposed in Proc. Imp. Acad. Tokyo, 9, 1935, may be applicable only to the former group. Full investigation in connection with genetics is now underway.

Parker, D. R. Locus of wy^2 (formerly cx_p).

Crossover counts on the male offspring of females $y\ v\ f/y^2\ wy^2\ g^2$ were made in order to determine the locus of wy^2 more accurately. The results are given: $v\ f$ - 1163; $wy^2\ g^2$ - 1111; $v\ wy^2\ g^2$ - 151; f - 126; $v\ g^2$ - 27; $wy^2\ f$ - 38; v - 208; $wy^2\ g^2\ f$ - 180; $v\ wy^2\ f$ - 1; g^2 - 0; $v\ wy^2\ g^2\ f$ - 5; $+$ - 5; $v\ g^2\ f$ - 1; wy^2 - 1; $v\ wy^2$ - 1; $g^2\ f$ - 0; Total 3018.

These data place wy^2 about 2 units to the left of garnet. 100% of the F_1 females of a cross of wy^2 x wy were phenotypically wy; there was no crossing-over observed between wy and wy^2 in 1328 offspring from wy/wy^2.

Stark, M. B. Varieties of tumors.

Selected stocks heterozygous for lethal-7, where the 1-7 males die from the development of melanotic growths, show that the tumors occur in characteristically different tissues. A preliminary description of the stocks follows:

& \$ 1 Carcinoma or melanonoma of salivary gland
& \$ 2 " " " of stomach region
& \$ 3 " " " of lower intestine
& \$ 4 Lympho-sarcoma
& \$ 5 Pigmented lipoma

The third-chromosome "benign" tumor is found to involve connective tissue.

Stone, Wilson. Allomorphic phenomena.

y^{35a} An allele, phenotypically like y^1, induced in the inversion, 99b, by x-rays.

y^{31e} (y^{303h}) A mutation accompanying a long inversion, probably y^{3P} as designated by Muller, for it gives the same males hypoploid for y and ac by crossing-over with sc^8. This mutation is between y^2 and gray in phaenotype, but gives occasional patches of y^1 bristles and microchaetes.

y^{31d} sc^8 w^a(5 k). An x-ray induced allele in sc^8, phaenotypically like y^2.

The following crosses differentiate between these alleles:

Cross			Phaenotype of F_1 ♀
y^{35a} 99b	x	y^1	y^1
y^{35a} 99b	x	y^2	gray $(\not+)$
y^{35a} 99b	x	y^{31e} (303 h)	31e with y^1 spots
y^{35a} 99b	x	y^{31d} sc^8 w^a	y^2
y^2	x	y^1	y^2
y^2	x	y^{31d} sc^8 w^a	y^2
y^2	x	y^{31e} (303 h)	y^{31e} -- no y^1 spots,

for in y^2, all bristles and microchaetes are black. y^{31e}/y^2 appears darker than y^2/y^1, although this could not be determined accurately, since y^{31e} is so nearly normal in color.

Although y^{35a} and y^1 are phaenotypically alike, as are also y^2 and y^{31d}(sc^8 w^a), their gene action is not identical for all steps of pigment formation. Thus, the action of these yellow alleles show them to be qualitatively as well as quantitatively different.

f^{34b}: An allele of forked, induced by x-radiation, which is phaenotypically normal in both males and females. When crossed to the original forked f, it gives a few weakly forked bristles, but shows more pronouncedly when crossed to f^5. (f^5 seems to be an amorph (Muller)). This allele is not a hypomorph, but is not strongly hypermorphic, since one dose is not sufficient to compensate for an absence and form normal bristles in the f^{34b}/f^5 condition, although it often does so in the f^{34b}/f heterozygote. It is interesting to note that the action of f^{34b} is equivalent to the "position effect" action induced in the normal allele of cubitus-interruptus by translocation involving chromosome 4, as found by Dubinin and Sidorov.

Timofeeff-Ressovsky, N.W.
Induction of mutations by alpha-particles in D. melanogaster.

The penetration-power of alpha-particles of radium is so low that it is impossible to induce mutations by external irradiation of flies. Thus, the following method was employed with sucess: The flies were put in a bottle with a cork-stopper containing a radonator. The flies (males) were breathing the emanation produced by the radonator for about 24-40 hours. After this treatment, the males were mated to ClB-females, and in F_2 the per cent of sex-linked lethals and visibles was determined. The mutation-rate was 1% in the treated series, as compared with 0.12% in the controls, the difference being statistically significant. Exact measurements of the radioactivity of the treated flies showed that the energy of the alpha-particles, emited by the emanation and the radioactive precipitate in the fly-tissues, is equivalent to an X-ray dosage that would produce about 1.5% of sex-linked mutations. The energy of the beta-particles and gamma-rays within the same flies is so low (about 1% resp. 0.01% of the alpha-energy) as to be negligeable. Thus, the effect produced must be ascribed to the action of alpha-particles emitted by the radioactive precipitate in the tissues of flies breathing emanation of radium.

Gershenson, S. Possible role of the genetically-inert region of the X-chromosome in equational divisions. When bobbed-deficiency ($sc^4 - sc^8$) males are crossed with *yy* females, patroclinous females are found in the offspring with a much higher frequency than usual. It seems, therefore, probable that the genetically-inert region of the X plays a role not only in the conjugation of chromosomes during synapsis, but in the equational divisions as well. In two cases among seven, several patroclinous females were found in the offspring of one bb-def. male. Such a coincidence could hardly have been accidental and probably means that equational non-disjunction of the X's took place several cell-generations before maturation.

Technical Notes

Beadle, G. W. Collection of eggs. For the collection of eggs for measures of egg or larval-pupal mortality, small paper spoons containing food have commonly been used. They have the disadvantages of giving a food mass of unequal thickness and usually with a rounded surface. Detection of all the eggs is often difficult. Small nickel boxes made of sheet material about 0.3mm. thick and of the dimensions 15 x 40 x 4mm. with a strip 45 x 10mm. soldered to the bottom so as to project about 30 mm. have been found to be very useful for egg counts and for collecting larvae of known ages. Standard cornmeal agar (containing animal charcoal, if desired, to increase the contrast) is pipetted into these boxes, filling them level full. They can be used in 20 x 100 mm. vials very conveniently. Examinations under a binocular can be made very rapidly. Experiments with different media with and without yeast indicate that yeast is a very important factor in stimulating rapid egg-laying. Standard food "painted" with a rather heavy suspension of yeast gives very satisfactory results. If it is necessary to have the eggs develop into adults, it is easy to slide the food mass out of the box on a cardboard strip 9 x 70 mm. It can then be transferred with eggs or larvae to a standard culture bottle containing food. With care, no eggs or larvae need be lost in the transfer.

Christie, A. L. M. Culture conditions for D. Subobscura. D. subobscura is being worked with in this laboratory and at first considerable difficulties were experienced with the culture conditions. The flies were reared in a 20-22 C incubator and on the usual D. melanogaster food medium. Of 200 single pair matings set up, 92 were sterile. The fertile matings gave on an average about 166 flies during a counting period of 19 days. The development takes at this temperature between 19 and 21 days.

It was then found that rearing the flies in a 15 C incubator gave better results. Of 60 single pair matings set up, only 2 were sterile. The average number of flies per fertile bottle was 168 and the time for emergence, 28 days. The addition of 0.05% of Nipagin to the food was found successful in preventing the molds at the low temperature. Before being set up in the culture bottles, the flies have to be held in mating vials; the tests carried out have shown that the best results are obtained after the flies have been held in the vials for 5 days.

Parker, D. R. Method of carrying stocks.

The early method of carrying stocks in this institution was to keep them in bottles, merely shaking them from the old one into the new one at each change, with occasional etherization and examination of them. Last year, however, we adopted a new method which seems to be far more efficient. The stocks are now carried in vials, keeping one old vial and mating three new ones at each change. The four are fastened together by means of a rubber band to which is attached the tag label. The flies are etherized by means of the mass method of Altenburg.

The advantages of this system are: (1)The flies are examined at each change, and (2) by making 3 new vials the chances of loss by contamination are greatly reduced. It is possible by this method to practically rid all of the stocks of mites, provided there are no adverse conditions of temperature.

This method takes a bit more time than the older one, but it will perhaps repay the loss with better stocks.

Parker, D. R. Moldex-A as a mold inhibitor.

Tests were run recently to find a substance to inhibit the growth of mold. The compounds tried out were Moldex-A, Nipagin-M, and Nipagin-T. These were added to our regular banana food in the ratio of .15 grams of anti-mold substance to 100 c.c. of food. Twenty vials were made of each of the above compounds, as well as twenty vials of plain food.

One half of the vials were inoculated heavily with mold, and the other half left uninoculated. One pair of flies was placed in each vial. Moldex-A was the most efficient in the prevention of mold. However, in the uninoculated series, the Moldex vials gave a slightly lower yield of flies than did the plain food. Egg counts were then run to see the possible effect that Moldex might have on hatchability. Our of approximately 3000 eggs, 98.7% reached the adult stage. This is about 7% higher than the ususal hatch on plain food.

Not only is Moldex more efficient that Nipagin-T and Nipagin-M, but it has also the additional advantage of being much more economical. It may be obtained from the Glyco Products Co., 949 Broadway, New York, N. Y.

Schweitzer, Morton D. Collecting eggs.

During the past year various techniques of collecting eggs have been tried. The following method has regularly yielded 100-600 eggs per culture per four hour period, with an average of 300. Not infrequently, on the first day of collection, the yield has been as high as 800-1300 in a four-hour egg-laying period. (D. melanogaster, pseudo-obscura, and to a small extent affinis and miranda)

The important precautions to be observed for optimum yield of eggs are:

(a) The females should not be etherized at any time prior to use for this purpose.
(b) The medium should be seeded with yeast at least 6 hours and not over 24 hours before use.
(c) The surface of the medium should be slightly roughened just before being placed with the flies.
(d) The surface on which eggs are to be collected must be ventral to the flies.

The details of the procedure I have followed are as follows: Young flies, not over 24 hours old, are transferred to fresh food without etherization (20-40 ♀♀ and ♂♂). Two or three days later they are transferred to fresh food. At this time the medium on which the eggs are to be collected is prepared. It consists of ordinary cornmeal-molasses-agar with lampblack added to give contrast to the white eggs. The cornmeal is sifted before cooking. The food mixture is poured onto the ordinary type of paper milk bottle caps, leaving a margin of 1 cm. all around. When cool, the surface is uniformly seeded with fresh yeast. (Caps for 24 hours are prepared at one time.) The next morning the surface of the food on the caps is scraped with a metal tissue lifter. The flies are transferred to empty half-pint bottles which are capped with the prepared paper caps. The bottles stand with the caps down. New caps are substituted at appropriate intervals.

Eggs have been collected by this method continuously for a week or more at intervals of 2,4,6,8,12 hours. If the rate of oviposition falls off after a few days it may sometimes be renewed by transferring the flies to regular food bottles for 2-3 days. Strains that do not reach their optimum rate of egg-laying as early as the fourth day may be kept on regular food longer before beginning the experiment. (D. Pseudo-obscura does well after 7-10 days from hatching, affinis and miranda even later.)

If properly fitting caps are used (diam.=1.625" for Bridges-type bottle, and 1.640" for most others) they may be washed and reused indefinitely.

Schweitzer, Morton D. Handling eggs and larvae. When eggs are collected in the manner outlined above the usual high mortality due to handling and yeast overgrowth may be minimized by several precautions. After counting, the entire slab of food (or a segment containing an appropriate number of eggs) may be transferred to the surface of regular unyeasted food. If the surface of the food on the cap is sliced off with a scalpel just before use, the danger of yeast overgrowth is much reduced. An alternative method of transfer, that has given high percentages of imagines, is to allow the eggs to hatch of the food while it is still attached to the cap. The young larvae are transferred with a fine scalpel. In transferring larvae, an efficient method is to gently touch the scalpel to a larva, then touch the larva to a second one, etc. until 25-75 are adhering to each other. In this way the larvae are subject to a minimum of direct handling.

Research Notes

Bedichek, Sarah. Suppression of the recessive *plexus* by the dominant *Star* in the II chromosome of D. melanogaster.

In connection with crossing-over studies in a II-III translocation it was found that the expression of the recessive mutant *plexus*, located at 100.5, II, in the homozygous condition is completely suppressed by the presence of one dose of the dominant *Star*, located at 1.3 in the same chromosome. The data is obtained from a cross in which both *plexus* and *Star* are present in a II-III translocation. In this case, II L is joined with III R and III L with II R. The homozygous translocation containing *c px sp* in one of the II R's and *S* in one of the II L's was crossed to *al dp b pr c px sp*. In the progeny, normal, Star, *c px sp*, and *S c sp* flies were found. The *S c sp* flies were never px.

Camara, A. Branched chromosome structure.

Studying the salivary gland chromosomes of the stock "plexus" (D.pseudoobscura) obtained by high temperature, in order to find evidence of one inversion detected by genetical results, we observed in some individuals an interesting branched structure of the X-chromosome. It is clear that the end of the proximal part of the second chromosome was translocated to that one, being laterally attached near the distal end of the inversion.

Camara, A. Effect of centrifuging on crossing-over.

The effect of centrifuging was studied in Drosophila melanogaster in the 3 chromosome. The investigation is now complete. The results are summarized as follows:

ru-53-	h-31.4	th-3.6	st-8.0	p^p-4.0	cu-24	sr-	c^s
41.5	30.0	2.3	6.0	3.5	18	29.0	-
38.5	29.0	2.2	4.0	4.0	19	27.4	-

Camara, A. Induction of mutations by high temperature.

We intended to test the production of mutations in a series of alleles. We started with purple' (D. pseudoobscura) and obtained pr^2, pr^3 and pr^4. We started also with pr^3 and obtained pr'.

Crew, F. A. E. Developmental studies.

The development of the legs, wings and halteres in the larva and early pupa of D. melanogaster has been followed up with the aid of sections and total preparations. The development of several wing mutants - dp, 13d (Jollos), vg - has been studied as to the first deviation from normal. The wing obtains its shape by the obliteration of marginal parts after the formation of the pupa sheath. The narrow wing of 13d shows its first deviation from normal in the first hours after pupation when

The circular portion of the wing disc (cf. Chen '29) grows out into a hollow pouch. In vg the circular portion is markedly smaller than in wildtype. As soon as it begins to grow out - or even earlier - the future proximal portion of the wing is constricted off by a fold. Later this portion becomes obliterated (cf. Goldschmidt '35). For the mutant 17b (unequal wings, Jollos) a temperature-effective period at the end of the larval and beginning of the pupal period was established.

Studies on the early development of vg, on the development of unequal wings and on the development of venation are in progress.

Pupae of wildtype, seven mutant eye-color types, and various combinations of mutant types of D. pseudo-obscura were isolated within one hour of pupation. After incubation at 25° C. for definite periods these were dissected and records kept of times at which pigment first appeared and times at which color changes occurred in the developing eyes. Histological studies of all important stages thus determined are now in progress.

Kichijo, H. Salivary chromosomes of various species. According to Kikkawa's suggestion, the ratio of the total length of autosomes to that of X-chromosome in the salivary gland cell of various species, was examined. The following 15 species gave the ratio about 4:1; melanogaster (A-type according to Metz and Moses' diagram), simulans (A), takahashii (A), immigrans (D), virilis (F), funebris (G), repleta (I), hydei (I), ananassae (L), bipectinata (L), montium (new type), sp-1 (A), sp-2 (A), sp-3 (A), and sp-4 (H). The following four species gave the ration about 1.7:1; pseudoobscura (J), affinis (K), sulcata (new type) and sp-5 (E). Full investigations in connection with the genetics and morphology are now under way in collaboration with H. Kikkawa and F. T. Peng.

Kikkawa, H. Chromosomes of D ananassae. As shown previously, four pairs of the V-shaped chromosomes are seen in the oogonial metaphase of this species. However, the linkage groups to date are only three: X-25 (including multiple allels), II=16, III=11, IV=0. This fact strongly suggests that one pair of the germinal chromosomes are formed by inert substances. Recent studies on both genetical and cytological grounds proved clearly that the smallest pair of the V-shaped chromosomes was almost inert. The most interestin point is that the distal part of one arm of this inert chromosome is homologous to a part of the short arm of Y-chromosome. The detail will be shown before long.

Ludwig, M. Asymmetrie-Index bei Crossover-Versuchen. Erhält man z.B. in einem 3-Punkt-Versuch abc/+++ x abc die Nachkommen (0)=466+682, (1)=39+8, (2)=113+215, (3)=225+322, (1,2)=7+2, (1,3)=20+23, (2,3)=50+125, (1,2,3)=15+1, Total 2312, so addiere man die kleineren Zahlen jeder Bruchklasse (466+8+113+225+2+20+50+1=885) und die grösseren Zahlen (682+39+215+322+7+22+125+15=1427), dividiere die grössere Summe durch die kleinere (1427:885=1,61), dann ist J = 1,61 der Asymmetrie-Index des Versuchs. Für die Berechnung von exakten Recombinationswerten, besonders für die Berechnung für Coincidence-Werte sind Versuche mit J=1,00-1,15 einwandfrei, J=1,15-1,30 brauchbar, J=1, 30-1,50 unsicher (kaum brauchbar), J grösser als 1,50 unbrauchbar. Dies ergibt sich praktisch und ist theoretisch begründbar. Falls in einem Crossover-Versuch die Teilklassen nicht einzeln angegeben sind, sollte immer mindestens der Asymmetrieindex angeführt werden.

Neuhaus, M. A New Allel of the Gene Yellow in Drosophila melanogaster.

In studying the effect of X-ray quality on the frequency of visible mutations (Bx males treated and mated with XX females), a gray male with yellow bristles was found and crossed to an XX female, resulting in F_1 XX daughters and gray males with yellow bristles, indicating that this character is probably sex-linked. The F_1 males were crossed to Florida females. In F_1 only Bx females appeared, showing the recessive nature of this character. Males having this trait were crossed to two groups of females with free X's. One group carried the gene for yellow skin and yellow bristles and the other group carried the gene for yellow skin and the genes w f. The former gave gray Bx daughters with yellow bristles in F_1. The latter gave only Bx daughters. It may therefore be concluded that the gene for this trait is partly allelomorphic to yellow. From F_1 females of the second cross, F_2 was obtained. This was done for the purpose of localizing the gene for the above character by the crossing-over method. F_2 gave the following results: 0:901; 1:8; 2:642; 3:23; 1-2:1; 2-3:13.

These data show that the new gene is located on the left end of the X-chromosome and is linked with a slight suppressor of crossing-over.

Shapiro, N. Comparison of the frequency of translocation between the 2nd and 3rd chromosomes in spermatozoa containing the X-chromosome and the Y-chromosome.

Our previous findings on translocations occuring under the influence of x-ray treatment have enabled us to collect some data concerning the translocation rate in spermatozoa containing the X-chromosome and those containing the Y-chromosome.

There are reasons to assume that:

1. The configuration and mutual position of chromosomes during treatment has an influence on the character of the translocation process.

2. The configuration and mutual position of chromosomes in nuclei of spermatozoa carrying the X-chromosome may differ from that in spermatozoa carrying the Y-chromosome.

Since one is inclined to admit these assumptions, our data can be of interest.

We have studied the translocation rate between the 2nd and 3rd chromosomes. The total of 915 pairs of treated 2nd and 3rd chromosomes was examined. 433 pairs were treated in spermatozoa containing the X-chromosome, and 482 pairs in spermatozoa containing the Y-chromosome. The percentage of translocations in the former was equal to 8.31%, while in the latter it made 8.29%. It is obvious that the translocation rate between the 2nd and 3rd chromosomes is equal in both cases.

Steinberg, Arthur G. The effect of inversions in the autosomes on crossing-over in the X-chromosome. The Cy inversion containing inversions in both arms of the second chromosome and the Payne inversion bearing inversions in both arms of the third chromosome were used. Counts were made on the ♂ offspring of ♀'s heterozygous for alternated xple and either one or both of the inversions. - Autosomal inversions cause an increase in crossing over in the X chromosome. This increase is greatest in the left end of the chromosome and decreases toward the right end. The increase is due to a decrease in non crossover strands and an increase in double and triple crossover strands. The per cent of single crossover strands recovered remains statistically constant. - These results are similar to those obtained by Miss H. Redfield for the second and third chromosomes.

Stern, Curt and Dorothy Doan. Crossing-over in the male between X- and Y-chromosome. In experiments involving the theta duplication (Muller) which is attached to the fibre end of the chromosome, individuals were found suggesting loss or detachment of theta. A preliminary genetic and cytological analysis (Spring 1934) demonstrated the occurrence of crossing over in males between the X or theta and the Y-chromosome as the cause. The detached theta and the detached X-chromosome carry complimentary arms of the Y-chromosome attached to their fibre ends. - A further analysis is at present carried on by D. Doan.

RESEARCH METHODS

Construction and maintenance of stocks

Note by editors The material by H. J. Muller for the following sections was prepared in the form of pictorial diagrams on large wall charts, and was thus exhibited by him at the Sixth International Congress of Genetics at Ithaca in 1932. Photographs of these charts were supplied by Muller as the material for the following accounts. In order to reduce the charts to forms suitable for reproduction by typewriting on stencils, the editors have transcribed them, with as few as possible changes in the basic plan of each chart. With respect to details, they have made minor changes, to wit:- (1) recasting the nomenclature current in 1932 into that of 1935, (2) employing "f" as the marker for a "foreign" chromosome to be introduced into a given "homozygous" or "host" stock, which is marked by "h", and (3) substituting Pm for S , and ey^D for ey, as markers. (Star can not easily be told in flies with other eye shapes, while Plum is excellent; the use of ey left the emergent stock encumbered with this character, while the use of dominant markers (ey^D and ci^D, available since 1932) for chromosome 4 gives a clean stock.

Muller, H. J. Construction of Homozygous Stocks. (See also similar technique by Bridges, Carn. Inst. Wash. Yr. Bk. 28:338, 1929)

P_1 ♀^{h} = donor for desired homozygote (or donor may be ♂; if so, mate to $\underline{y};Pm/+;H/+$ ♀)
X
$Pm/+;H/+$ ♂♂ (Dominant markers in 2 and 3 may be replaced by T(2-3) with one dominant marker).

F_1 one ♂ $1^h;Pm/2^h;H/3^h$ (save for remating !) X $C\underline{l}B/+;Cy/+;CC,D/+$ ♀♀ (different dominant markers)

F_2 Father (remated) X $C\underline{l}B/1^h;Cy/2^h;CC,D/3^h$ ♀♀ (crossing-over prevented)

F_3 $1^h;2^h;3^h$ completely homozygous ♀♀ X ♂♂

For controlling 4 also, the 4th chromosome dominant ey^D may be used together with $Pm/+;H/+$ (or with $\underline{y};Pm/+;H/+$), and ci^D with $ClB/+;Cy/+;CC,D/+$. For controlling Y also, mate donor ♂ to $C\underline{l}B/+;Pm/+;H/+;ey^D/+$ ♀ ; save donor ♂ and remate to $C\underline{l}B/1^h;Pm/2^h;H/3^h;ey^D/4^h$ daughter; use one son ($1^hY^h;Pm/2^h;H/3^h;ey^D/4^h$) to mate to $C\underline{l}B/+;Cy/+;CC,D/+;ci^D/+$ ♀♀; save him and proceed as above for F_2 and F_3.

F_3 yields also all possible combinations of chromosomes of P_1 donor, homo- and heterozygously, and distinguishable by markers, for analysis of contributions of all the chromosomes (whether recessive or dominant) to multiple factor effects, and for tracing sources of variability. F_3 reveals also all recessive lethal and visible mutant genes of P_1.

Chromosomes of desired "h" stock need not carry any visible mutant.

Muller, H.J. Insertion of Foreign Chromosome into Homozygous Host Stock

(Stocks f and h need not contain any visible mutants)

(1) For substituting 1^f for 1^h in $1^h;2^h;3^h;4^h$ stock
P_1 f ♂♂ X ClB/+;T(2-3)S Cy/+,+;cy^D/+ ♀♀
F_1 1^f/ClB;2^f,3^f/T(2-3)S Cy;4^f/cy^D ♀♀ X h ♂♂
F_2 one ♂ 1^f;2^h,3^h/T(2-3)S Cy;4^h/cy^D X ClB/+;Cy/+;CC D/+;ci^D/+ ♀♀
(save for remating!)
(Different markers; Inversions)
F_3 Father (remated) X 1^f/ClB;2^h/Cy;3^h/CC,D;4^h/ci^D ♀
F_4 $1^f;2^h;3^h;4^h$ completely homozygous ♀♀ X ♂♂

(2) For substituting 2^f for 2^h in $1^h;2^h;3^h;4^h$ stock
P_1 f ♀♀ X Pm/+;H/+;cy^D/+ ♂♂ AND h ♂♂ X ClB/+;Cy/+; CC,D/+;ci^D/+ ♀♀
F_1 1^f;2^f/Pm;3^f/H;4^f/cy^D ♂♂ X 1^h/ClB;2^h/Cy;3^h/CC,D;4^h/ci^D ♀♀
F_2 one ♂ 1^h;2^f/Cy;3^h/H;4^h/cy^D X 1^f/ClB;2^f/Cy;3^f/CC,D;4^f/ci^D ♀♀
(Recombination; save!)
F_3 Father X 1^h/ClB;2^f/Cy;3^h/CC,D;4^h/ci^D ♀♀
F_4 $1^h;2^f;3^h;4^h$ completely homozygous ♀♀ and ♂♂

(3,4) Analogous schemes apply for 3 and 4 (selecting appropriate F_2 ♂)

If desired, foreign autosome may first be made like that in homozygous stock except in regard to particular region. Method for this is shown below in scheme for combining invisible genes in the same chromosome.

Muller, H. J. Insertion of Desired Genes into Attached X's

P_1 y ♀♀ X T(1-4)B^S ♂♂ (Austin T; left part X attached to 4; rt end X carrying "Bar-super")

F_1 y/XR,B^S ♀♀ X m g^2 sd f ♂♂ (Let m, g^2,sd, f be the genes to be inserted)
F_2 (Crossing-over) y·B^S/m g^2 sd f ♀ X m g^2 sd f ♂♂
F_3 (Crossing-over) m g^2 sd f·B^S /m g^2 sd f ♀ X + ♂♂
F_4 (Crossing-over) m g^2 sd f Y ♀

In earlier method, used by L.V. Morgan et. al, genes were inserted by crossing-over, from separate X-chromosome containing them, into attached X's, in triploids.

Muller, H. J. Combination of Invisible Genes

Nearly same methods apply for getting desired chromosome combinations of recessives that always must be worked with in the heterozygous condition.

1. Two invisibles in different autosomes (a in 2; b in 3)
P_1 a/2 ♂ X S/+;CC,D/+ ♀ AND b/3 ♂ X Cy/+;CC,Dfd/+ ♀
F_1 a/S;CC,D/+ ♀ X Cy/+;b/CC,Dfd ♀
F_2 a/Cy;b/CC,D ♂ X a/Cy;b/CC,D ♀
(balanced stock, if recessives have low viability or low fertility)
F_3 a/a;b/b ♂ and ♀ (if homozygotes are viable and fertile)
(for chromosome 4, markers ciD and cy^D may be used in same way.

2. Getting combination of invisibles in 1 and 2 (a in 1;b in 2)
P_1 a/1 ♀ X Cy/+ ♂ AND b/2 ♂ X ClB/+;Pm/+ ♀.
(double mated) y;Pm/+ ♀
F_1 a;Cy/+ ♂ (double mated) X ClB/+;b/Pm ♀ and Xy;b/Pm ♀
F_2 a/ClB;b/Cy ♀ X a;b/Cy ♂ (balanced Ø)
F_3 a/a;b/b ♀ and a;b/b ♂ (if homo. viab. and fert.)

Analogous scheme using CC,D and CC,Dfd or Mc-2 for 3 applies for chromosomes 1 and 3.

3. Getting combination of two invisible genes (a and b) in same chromosome (in known positions). Let map order be a, v_1, v_2, b where v_1 and v_2 are visible genes between a and b.
P_1 a/+ X v_1 v_2 AND b/+ X v_1 v_2
F_1 a++/+v_1 v_2 ♀ X v_1 v_2 ♂ AND ++b/v_1 v_2 + ♂ × v_1 v_2 ♂
F_2 (by crossing-over) a + v_2/+ v_1 v_2 X v_1 + b/v_1 v_2 +
(chance of losing a or b from ♀ obviable by interpolating cross of ♂ by individual containing inversions)
F_3 a + v_2 +/+ v_1 + b ♀ X v_1 v_2 ♂
F_4 (by crossing-over) a++b/v_1 v_2 X D_1 v ♀
(where D_1 is dominant linked and where v is either v_1 or v_2)
F_5 a b/D_1 v ♂ X C,D_2 ♀ (where D_2 is a different linked dominant)
(lethal when homozygous, and C is an inversion preventing crossing-over).
F_6 a b/C,D_2 ♂ and ♀ (balanced Ø)
F_7 a b/a b ♂ and ♀ (homozygous Ø)

Combination of one invisible with one visible is correspondingly simpler.

Muller, H. J. and C. B. Bridges
Balanced Stocks

Given "1", a gene (or combination of linked genes) with low or no viability or fertility when homozygous in ♂ or ♀ (or both, and "C" a genetic complex that is non-perpetuating or lethal when homozygous, that contains gene rearrangements which prevent crossing-over between "1" and "C" and that preferably contains a dominant marker, then the most usual balanced stock for maintenance of "1" without selection is:

1/C ♂ X 1/C ♀

1/1 (fails to perpetuate); C/C (dies); 1/C and C/1 (continue as before.

To construct simple balanced autosomal stocks (1/C)
Given 1 a/+ (or 1/a) ♂, where a = recessive marker and M = a dominant linked marker subject to crossing-over. Then:
P_1 M/+ X a/a
F_1 M/a ♀ X a/a ♂
F_2 M a/a ♀ X 1 a/+ ♂ (or 1/a ♂)
F_3 M a/1 a ♂ X C/+ ♀ (or M a/1 X C/+ ♀)
F_4 1 a/C ♂ and ♀ (or 1/C ♂ and ♀)

The more used complexes represented above by "C" are as follows:

Chromosome	Complex
1	ClB (Bar eyes), very little crossing-over. (Muller)
"	dl-49, sc lz^s (only recessive markers), crosses over right of garnet (Muller)
"	dl-49, y Hw (Hairy wing), crosses over right of garnet (Muller, Redfield)
2	Cy (Curly wings), with 2 inversions and very little crossing-over (Ward)
"	C2L C2R sp (only recessive marker, speck), 2 inversions, some crossing-over in middle. (Sturtevant)
"	Pm (Plum eye) inversions involving 2L and 2R. (Muller)
3	CC, D (Dichaete) little crossing-over except near ends (Oliver, Stone)
"	CC, Dfd (Deformed eye) 2 inversions, moderate crossing-over in middle (Payne, Bridges)
"	C3X, M3X (Minute bristles) 2 inversions, moderate crossing-over in middle (Muller)
"	Mo-2 (Moire eye) inversions and translocation with 2, crossing-over only near right end (Muller)
2 & 3	T(2-3) S Cy (Star eye, Curly wings) inversions and translocation 2-3, moderate crossing-over (Muller)
4	ci^D (Cubitus-interruptus - Dominant - venation) (inversion not needed for 4) (Sturtevant)
"	ey^D (eyeless - Dominant) (inversion not needed for 4) (Muller)

Many other usable complexes, some lacking dominant markers and many affecting a limited part of a chromosome, are avialable. (See list of symbols in DIS-3).

Muller, H. J. To Balance Sex-linked Genes

1. General method for genes to left of garnet.

Symbols:

1 = sex-linked gene or combination of genes with low or no viability or fertility in ♂, or else in homozygous ♀.
sc = scute
lz^s = lozenge-spectacled (sterile in homozygous ♀, not in ♂)
dl-49 = inversion (left break about 11, right about 44 on map)

P_1 1/sc ♀ X dl-49, sc lz^s ♂
F_1 1/dl-49, sc lz^s ♀ X dl-49, sc lz^s ♂
F_2 1/dl-49, sc lz^s ♀ - like mother, fertile and continues ∅
dl-49, sc lz^s/dl-49, sc lz^s ♀ - sterile
1 ♂ - dies or is sterile or yields lethal or sterile 1/1 daughter
dl-49, sc lz^s ♂ - like father, fertile and continues ∅

2. Genes to right of garnet may be balanced similarly, using CR ♀s B in place of dl-49, sc lz^s. CR is rearrangement preventing crossing-over to right of garnet, ♀s makes homozygous ♀ sterile (but not ♂) and B is Bar eye.

3. If "l" is itself associated with a rearrangement preventing crossing-over, then "C" in chromosome balancing it is unnecessary. Example:
   Cl/lz^s ♀ X lz^s ♂ is balanced stock, though lz^s here is in normally arranged chromosome instead of with dl-49 inversion.

4. Other genes like lz^s which do not kill or sterlize ♂ but only homozygous ♀, may be balanced also with ClB. Example:
P_1 ClB ♀ X sn ♂ (sn = singed; sn ♂ fertile; sn/sn ♀ sterile)
F_1 ClB/sn ♀ X sn ♂ (balanced stock)
F_2 ClB/sn ♀ (fertile) sn/sn ♀ (sterile) ClB ♂ dies
sn ♂ fertile

5. Genes in class 4 also may be balanced against attached X's
y ♀ X sn ♂
y/sn ♀ (3X's - sterile) y ♀ sn ♂ no-X(dies)

Method 5 is inferior to others whenever ♀ ♀ with "l" may be required.

Methods 4 and 5 are inferior to 1 and 2 where "l" ♂ is hard to obtain or to breed.

Austin, Tex. laboratory Balancers Chromosome 1: ClB - Good, except for extreme ends.

sc^8 sn - good for chromosomes not crossing-over in the middle region (e.g. Translocations &c)
99b sn - Ditto.
dl-49 - Balances middle region.
In-Am - Balances right end (f-bb) (probably left as far as y)
Chromosome 2:
Cy - Balances all of chromosome; Crosses over but seldom.
Pm -
NS -
Chromosome 3:
CMc - Inversion in left arm
T2,3-C c - Translocation with inversions; balances all of chromosome except the ca region.
Dcx - Double inversion including D; balances middle of chromosome (all of chromosome except ru-h, & ca)
C3X - Balances most of chromosome, but crosses over too frequently.
C3c -
Payne -

Oliver, C. P. Balancers X-chromosomes with genes which cause sterility or poor viability in males and with which ClB cannot be used, can be balanced with the Dl inversion (Muller's) that has connected with it the visible spectacle eye. The spectacle female is sterile. Only a small per cent of crossing-over occurs to the left of forked. If spectacle-forked males are used to balance, no or only occasional selection is required. Punch eye, dominant, is a useful balancer for inviable genes in the left arm of 3 except for a small amount near the left end. The viability of punch is good.

Zuitin, A.I. A Third Chromosome Balancer

The lines carrying numerous mutant genes in the third chromosome may be balanced by means of the chromosome ru h D (Fiodorova) carrying the inversion which almost completely suppresses crossing over (to be described in one of the following issues of C.R. Ac. Sc. U.S.S.R.). The stock is : ru h D inv/ru cu ca; Cy sp/L^2.

Muller, H.J. Labor-Saving Method of Starting Homozygous or Balanced Stocks of ♂-fertile Sex-linked Genes

(1) Balancer stock "Patroclinous" ("Pat")
P$_1$ ClB, sc v/sc v lx f bb/Y ♀ (non-disjunction) X dl-49, lz^s/Y ♂
Eggs: ClB, sc v (half carry Y) sc v lx f bb (half carry Y)
ClB sc v/sc v lx f bb (non-disjunction) Y (non-dis.)
Sperm: lz^s dl-49 Y
F$_1$ ClB, sc v/dl-49, lz^s ♀ (1/2 carry Y) ClB, sc v/Y (1/2 YY) (die)
sc v lx f bb/dl-49, lz^s ♀ (" " ") sc v lx f bb/Y ("" ")
ClB, sc v/sc v lx f bb/dl-49, lz^s ♀ (dies) ClB, sc v/sc v lx f b 'Y ♀
dl-49, lz^s/Y ♂ Y/Y (dies)

To perpetuate, breed sc v B ♀♀ (need not be virgin) by dl-49, lz^s brothers or fathers (Selection needed to retain sc v lx f bb).

(2) Let "a" represent sex-linked gene or combination of genes. Then:
P$_1$ ClB, sc v/sc v lx f bb/Y ♀ X a/Y ♂
Eggs: ClB, sc v (1/2 carry Y) sc v lx f bb (1/2 carry Y)
ClB, sc v/sc v lx f bb (non-disjunction) Y (non-dis.)
Sperm: a Y
F$_1$ ClB sc v/a ♀ (1/2 carry Y) ClB, sc v/Y ♂ (1/2 YY) (dies)
sc v lx f bb/a ♀ (" " ") sc b lx f bb/Y ♂ (1/2 YY) (dies)
ClB, sc v/sc v lx f bb/a ♀ ClB, sc v/sc v lx f bb/Y
(3X ♀, dies or sterile) Y/Y (no - dies)
a/Y ♂

To form stock of "a", breed B (not sc or v) ♀♀ (need not be virgin) by any brothers or fathers: ClB, sc v/a ♀ (1/2 carry Y) X a ♂.
If "a" has good viability and fertility when homozygous, this stock will automatically become a/a/(Y) ♀ X a/Y/(Y) ♂ after a few generations; otherwise, it will remain balanced and this will then be the more desirable condition to have it in. If presence of supernumerary Y in stock is undesirable, a stock of "Patroclinous" should be used in which the Y is marked by Cy owing to a translocation between Y and a chromosome 2 containing Cy. In this case only the non-Cy B F$_1$ ♀♀ are used for breeding. This stock is called "Curly Pat".

Muller, H.J. Balancing of Duplications by Deficiencies or Lethals and Vice versa -(Bridges, Muller)

Example: P$_1$ y w/sc^{j1} lj1 ♀ X y/del-24/Y ♂ (del-24 is a deleted-X = duplication covering locus of lj1, y and sc).
F$_1$ sc^{j1} lj1/del-24/Y ♂ X y ♀

Sperm: sc^{J1} lJ1/del-24 : sc^{J1} lJ1/Y : sc^{J1} lJ1 : del-24/Y : del-24 : Y

Eggs	y	Y
F$_2$	y/sc^{J1} lJ1/del-24 ♀ (sterile)	Y/sc^{J1} lJ1/del-24 ♂ (fertile)
	y/sc^{J1} lJ1/Y ♀ (sterile)	Y/sc^{J1} lJ1/Y ♂ (dies)
	y/sc^{J1} lJ1 ♀ (sterile)	Y/sc^{J1} lJ1 ♂ (dies)
	y/del-24/Y ♀ (fertile)	Y/del-24/Y ♂ (dies)
	y/del-24 ♀ (fertile)	Y/del-24 ♂ (dies)
	y/Y ♀ (fertile)	Y/Y (dies)

Fertile F$_2$ ♀♀ bred with fertile F$_2$ ♂♂ give same classes as before; hence stock is now self-perpetuating.

Muller, H.J. Detection of Mutations

1. Visibles arising in X of ♂: (Cross ♂ to y/Y ♀ and examine sons)

P$_1$ y/Y ♀ X X/Y ♂ (donor)
F$_1$ y/X ♀ (3X, sterile) y/Y ♀ X/Y ♂ (examine) Y/Y (dies)

2. Lethals and visibles arising in X of ♂ (ClB method)

Key: B = Bar eye; C = inversion preventing crossing-over; n = new mutant, lethal, or visible; sc v f and car are recessive markers.

P$_1$ sc v f car/Cl B, sc v ♀ X X/Y ♂ (donor)
F$_1$ ClB, sc v/n? ♀ X sc v f car ♂ (breed in individual ♀ cultures)
F$_2$ sc ClB v/sc v f car ♀ (discard) ClB, sc v/Y ♂ (dies)
n?/sc v f car ♀ n?/Y ♂ (if lethal, dies)

Let "n" represent possible newly arisen mutant gene (lethal or visible). No crossing-over in F$_1$ ♀, because of inversion. If there is no "n" in given culture, ♂♂ will appear +. If there is a visible "n", all ♂♂ will manifest it. If there is a lethal "n", no ♂♂ will hatch (except occasional non-disjunctional sc v f car); this is determinable without etherization. If "n" proves to be present in a culture, make mass culture of F$_2$ n/sc v f car ♀♀ X brothers (visible), or, if "n" is lethal X sc v f car ♂♂; either way provides stock of "n" and count of F$_3$ ♂♂ shows locus of "n".

3. Visibles arising in X of ♀ are directly evident in sons.

4. Lethals arising in X of ♀

lz – lozenge-spectacled eye, sterile in homozygous ♀
dl-49 – inversion, preventing crossing-over to left of garnet.

P1 +/+ ♀ (donor) X dl-49, lz^{s} B/Y ♂
Obtain virgin F$_1$ ♀♀ and breed in individual ♀ cultures to dl-49, B ♂♂ (cross by brothers would yield some + F$_2$ ♂♂ by non-disjunction.) If l is present, no + ♂♂ appear; determinable without etherization. (If l is to right of garnet, a few + ♂♂ appear).

F$_1$ l/dl-49, lz^{s} B ♀ X dl-49, lz^{s} B/Y ♂
F$_2$ l/dl-49, lz^{s} B ♀ l/Y ♂ (dies) dl-49, lz^{s} B/dl-49, lz^{s} B ♀ (lz^{s} B ♀ sterile) dl-49, lz^{s} B/Y ♂

If l is present, F$_2$ provides balanced stock of it unless to right of garnet.

5. Detection of mutations in autosomes same as method for getting homozygous stock. Method there given may be split for use with 2 alone or 3 alone.

Agol, I.J. New Method of Detecting Lethals in the X-chromosome.

Up to the present time Muller's method ClB has been used to detect lethals in the X-chromosome. At our laboratory we have adapted the method of y B sc^4 on the basis of the following considerations:

(1) y B sc^4 represents an inversion involving almost the whole length of the X-chromosome (from sc to bb) while the ClB inversion is far shorter (approximately from bi to Bx). Therefore y B sc^4 excludes the possibility of getting single cross-overs on both sides of the X-chromosomes.

(2) By the ClB method half of the chromosomes tested are lost, as only half of the progeny get the ClB chromosome in F_1; while by crossing the X-chromosomes tested (males) with homozygous y B sc^4 females nothing is lost.

(3) The y B sc^4 method facilitates the establishment of balanced cultures with lethals, because 1/y B sc^4 obtained in F_1 and inbred individually with their y B sc^4 brothers will give two categories of females: 1/y B sc^4 and y B sc^4/y B sc^4 which are easily distinguished one from the other, while crossing 1/ClB with any male gives two kinds of females, which it is impossible to differentiate phenotypically.

In case a new lethal has arisen in a cross with y B sc^4 we do not get maleless cultures in F_2, but all the males instead of being of two categories will be of one kind only, namely y B sc^4.

The method is used as follows. The chromosome tested (male) is crossed with a virgin homozygous female y B sc^4/y B sc^4. In F_1 all females are picked out and inbred individually with their brothers. If only one class of males is obtained in F_2, this indicates the presence of a lethal in the X-chromosome under investigation.

Muller, H.J. Accumulation of Mutations (Negativing of Natural Selection.

1. In chromosome 1 (possible in ♀♀ only) (Cs B method). Key: Cs is inversion preventing crossing-over in right portion of 1 and containing gene making ♂♂ sterile. B = Bar eye. Dl-49 is inversion, preventing crossing-over to left of g in 1. lz^s = lozenge-spectacled eye, sterile in homozygous ♀. w = white eye.

P_1 Cs,B/ dl-49, w lz^s ♀ X dl-49, w lz^s ♂♂ (many single-♀ cultures).

F_1 Cs,B/dl-49, w lz^s ♀ dl-49, w lz^s/dl-49, w lz^s ♀ (sterile)
Cs,B/Y ♂ (sterile) dl-49, w lz^s/Y ♂.

Verify presence of B ♂♂ in each F_1 culture as preliminary test. Then breed F_1 ♀♀ in mass cultures X brothers, keeping those from different P_1 cultures separate.

F_2 offspring as before and so on to F_n (balanced 0).

Final test: single ♀ from each culture of F_n is bred to her brothers. If lethal is present in Cs B chromosome, no B sons will appear in $F_n \neq 1$.

2. In chromosome 2 (using rotund as sterilizer)
rn – rotund wing; sterile in homozygous ♂ and ♀ (found by Glass)
sp – speck (a marker)
P_1 rn sp/Cy ♂ X S sp/Cy ♀ (in many single cultures)
F_1 rn sp/Cy (breed ♂♂ and ♀♀) (discard other types of F_1)
F_2 rn sp/Cy (breeds; balanced $\not{8}$) rn sp/rn sp (sterile
Cy/Cy (dies)

Make mass cultures of F_1, F_2, etc. to F_n corresponding to each P_1 culture. Therefore, $F_1 = F_2 = F_3 = \ldots\ldots F_n$

Preliminary test: Examine F_2 for presence and normality of homozygous rn to insure against lethal or visible present at start of experiment.

Final test: Test single ♂ from each F_n culture, making same cross as of P_1 ♂; breed F_{n+2} in same way as F_1 and ascertain presence and normality of homozygous rn in F_{n+3} as in F_2.

3. In chromosome 3 (using inserted piece of 2 as temporary lethal). Key: Homozygous chromosome 3 carrying duplicating piece of 2 from T(2–3) Pale is lethal in hyperploid having normal 2/2 but non-lethal in fly having one normal and one Pale-deficient 2. 2^- = chromosome 2 deficient for fragment translocated to 3. 3^{+2} = chromosome 3 bearing fragment of 2.

Balanced Stock: Cy/2^-; CC,Cfd/3^{+2}

P_1 Cy/2^-; CC,Dfd/3^{+2} ♂ X CC,D/H ♀♀ in many individual ♂ cultures.
F_1 (a) 2^-/+ ; CC,D/3^{+2} ♂♂ and ♀♀ (one mass "test culture" from each P_1)
(b) Cy/+ ; CC,D/3^{+2} ♂♂ and ♀♀ (one mass "stock cultures" from each P_1)
F_2 (a) Examine offspring of test cultures for presence of non-D flies (2^-/+ ; 3^{+2}/3^{+2}) indicating that no lethal was present in 3^{+2} of P_1 ♂. If lethal was present discard sister stock culture. (Possible visibles in 3 are discovered simultaneously).
(b) Cy/+ ; CC,D/3^{+2} – Continue breeding from cultures whose sister test cultures showed no lethal (i.e. produced non-D flies).
F_n Same as F_2 (b).

Final test: Test single ♂ from each F_n "stock culture" crossing it by ♀♀ from stock Cy/2^-; CC,Dfd/3^{+2}

F_{n+1}: Cy/+; CC,D/3^{+2} (in single-♂ cultures) X Cy/2^-/CC,Dfd/3^{+2}
F_{n+2}: (Select non-Cy, non-D, Dfd) 2^-/+ ; CC,Dfd/3^{+2} ♂ and ♀
F_{n+3}: Examine for absence of non-Dfd (2^-/+ ; 3^{+2}/3^{+2}) to discover if lethal is present. (Also examine to discover visibles).

4. In chromosome 2, with automatic selection of required offspring. Symbols: ab^2 = abrupt^2 (" parted"); homozygous ♂ sterile; mr = morula; homozygous ♀ sterile; "n" indicates possible newly arisen mutant; L^2 = dominant marker, Lobe eye.
P_1 ab^2 mr/L^2 ♂ X Cy/+ ♀ (many single-♂ cultures)
F_1 ab^2 mr/Cy ♂♂ and ♀♀ (Breed F_1 in mass cultures, corresponding to each P_1 male)

F_2 ab^2 mr/Cy (balanced ØØ; examine each to see that ab^2 mr is present)
F_n Continue mass cultures of each non-lethal line to F_n

Test ab^2 mr chromosome of one ♂ from each F_n culture by fly from tester balanced stock ab^2 L^2 mr/Cy as follows:
F_{n+1} Cy/ab^2 "n" mr ♂ X Cy/ab^2 L^2 mr ♀
F_{n+2} ab^2 "n" mr/Cy (breed ♂♂ and ♀♀; balanced Ø of "n"

Look for "ab^2 mr" appearing F_{n+3} flies. If absent, "n" is lethal. If abnormal, "n" is a visible. If present and normal, no detectable mutation has occurred.

Muller, H.J. Accumulation of Mutations in Given Sex

1. In 1 of ♀: see "Cs,B" method above.

2. In 2 of ♂: by use of "Blond", a mutual translocation of 1 and 2, viable when homozygous and containing a dominant marker, the Blond bristles. (Found by Burkart with Stern). Key: 1 carrying right end of of 2 and deficient for left end of 1 = Bld^{-1L+2R}. 2 carrying left end of 1 and deficient for right end of 2 = 2^{-2R+1L}. "all" = a series of 7 recessive markers scattered through 2.
P_1 Bld^{-1L+2R} ; 2^{-2R+1L}/Cy ♂ (many single - ♂ cultures) X
X ; Cy/"all" ♀
F_1 Bld^{-1L+2R} ; 2^{-2R+1L}/Cy(or " all") ♂ X X ; Cy/"all" ♀
(ballanced Ø). One mass culture and line from each P_1 ♂.
(other combinations die or are highly infertile; if in latter crossing-over occurs, it is revealed by genes of "all").

Preliminary test for preexisting lethals and visibles in each P_1 ♂, and final test for new mutants is made as follows:
P_1 and F_{n+1} Bld^{-1L+2R} ; 2^{-2R+1L} $\underline{1}$(?)/Cy ♂ (single ♂ from each F_n line) X sc v $\underline{1}$x f bb/C$\underline{1}$B, sc v/Y;S/Cy ♀
(virgin ♀♀ of "Curly Pat" stock)
F_1 and F_{n+2} Bld^{-1L+2R} ; 2^{-2R+1L} $\underline{1}$(?)/S (or Cy) ♂ (only ♂♂ which live) X Bld^{-1L+2R}/C$\underline{1}$B, sc v (or sc v $\underline{1}$x f bb) ; 2^{-2R+1L} $\underline{1}$(?)/Cy ♀
(Choose non-sc, non-v, Cy orthoploid ♀♀, need not be virgin).
F_2 and F_{n+2} Look for non-S, non-Cy ♀:
Bld^{-1L+2R}/Bld^{-1L+2R} ; 2^{-2R+1L} $\underline{1}$(?)/2^{-2R+1L} $\underline{1}$(?)
If absent, lethal is present; if they are abnormal (except Blond character) visible is present.

3. In 2 of ♂ and 2 of ♀ simultaneously; also in two 2's which alternate between ♂ and ♀. (By use of "abrupt^2", sterile in homozygous ♂, of "morula", sterile in homozygous ♀, and of "rotund", sterile in both; crossing-over to be prevented by use of homozygous C3G, or inversions in "ab^2 pr mr" chromosome.)
P_1 rn mr/ab^2 pr mr ♂ X ab^2 rn/ab^2 pr mr ♀
(single ♂ and ♀ in many individual cultures)
F_1 (and F_{3-5} etc.) rn mr/ab^2 pr mr (♀ sterile; ♂ breeds)
rn mr/ab^2rn (♂ and ♀ sterile) ab^2 pr mr/ab^2 pr mr (♂ and ♀ sterile) ab^2 pr mr/ab^2 rn (♂ sterile; ♀ breeds)

F_{2-4-6} etc. rn mr/ab^2 pr mr (only ♂ broods) rn mr/b^2 rn (sterile
ab^2 pr mr/ab^2 pr mr (sterile) ab^2 pr mr/ab^2 rn
(only ♀ broods)

Test for lethals and visibles in P_1 or F_n:

P_1 or F_{n+1} $\underline{l}$(?) rn mr/ab^2 $\underline{l}$(?) pr mr ♂ X Cy,pr/S ♀
(Same method used for testing ab^2 pr mr/ab^2 rn ♀)

F_1 or F_{n+2} (Mate Cy, non-pr together . (Mate Cy,pr togehter)
$\underline{l}$(?) rn mr/Cy,pr ♂♂ and ♀ . ab^2 $\underline{l}$(?) pr mr/Cy,pr♂&♀

F_2 or F_{n+3} $\underline{l}$(?) rn mr/$\underline{l}$(?) rn mr . ab^2 $\underline{l}$(?) pr mr/ab^2$\underline{l}$(?)
. pr mr

(Look for presence and . (Look for presence and
normality of rn mr flies . normality of ab^2 pr mr
in F_2 or F_{n+3}. flies in F_2 or F_{n+3}.

Stern, Curt Technique for obtaining large numbers of unfertilized females.

Following a request by the editors of DIS a description is furnished of a genetic method published in 1929, Zeitschr. Abstgsl. 51: 315-316. A stock of the following constitution is maintained (Pasadena, Cold Spring Harbor, Rochester and other laboratories): g^2 B $\underline{XY'}$ Y"/y Y". The males thus possess the long arm of the Y-chromosome (Y') attached to the X-chromosome and a Y-fragment (Y") consisting of the short Y-arm plus part of the long arm. Y' carries the factor (or complex) K_1, Y" carries K_2, both of which have to be present to permit male fertility. The females have attached X-chromosomes and the Y" fragment. The stock keeps constant without selection.

1) In order to obtain unfertilized females with attached X-chromosomes virgin $\underline{y}$ Y" females of the stock are mated to males from any normal stock. The F_1 females will be $\underline{y}$Y and the F_1 males XY". If the P-individuals of such a culture have been removed before the hatching of the F_1, all males present will be XY" and sterile. All F_1 females, in spite of the presence of their brothers, will be unfertilized accordingly.

2) In order to obtain unfertilized females without attached X-chromosomes, $\underline{XY'}$Y" males from the original stock are mated to virgin females from a normal stock (in order to exclude the accidental use of XXY females it is advisable to take short bristled females from a bobbed stock). The F_1 males being XY" will be sterile and the F_1 females (X$\underline{XY'}$) will be unfertilized again.

The original stock should occasionally be tested for the occurrence of the very rare cross-overs in the XY' Y" males which lead to the reconstruction of a normal Y-chromosome. Test method: Mate in one bottle 1 female and 1 male from the stock and add females from a bobbed stock. Test the sons of the bobbed females for fertility. If sterile, continue the stock from the offspring of the test culture.

Observations on eggs, larvae, pupae and flies

Beadle, G.W. Collection of eggs. For the collection of eggs for measures of egg or larval-pupal mortality, small paper spoons containing food have commonly been used. They have the disadvantages of giving a food mass of unequal thickness and usually with a rounded surface. Detection of all the eggs is often difficult. Small nickel boxes made of sheet material about 0.3 mm. thick and of the dimensions 15 x 40 x 4 mm. with a strip 45 x 10 mm. soldered to the bottom so as to project about 30 mm. have been found to be very useful for egg counts and for collecting larvae of known ages. Standard cornmeal agar (containing animal charcoal, if desired, to increase the contrast) is pipetted into these boxes, filling them level full. They can be used in 20 x 100 mm. vials very conveniently. Examinations under a binocular can be made very rapidly. Experiments with different media with and without yeast indicate that yeast is a very important factor in stimulating rapid egg-laying. Standard food "painted" with a rather heavy suspension of yeast gives very satisfactory results. If it is necessary to have the eggs develop into adults, it is easy to slide the food mass out of the box on a cardboard strip 9 x 70 mm. It can then be transferred with eggs or larvae to a standard culture bottle containing food. With care, no eggs or larvae need be lost in the transfer. (Copied from DIS-4:64)

Marshak, A. Collection of eggs. Modification of Beadle's method for collecting eggs. Instead of using the metal containers for the agar-bone black medium, as suggested by Beadle, I have used strips of wood which can be easily cut and grooved with a buzz saw. The ends are then closed with plastic wood and the whole affair paraffined. The groove is filled with the agar medium till flush with the top and either seeded with yeast or moistened with acetic acid, and placed in a shell vial that is slightly longer than the wood strip. Egg counts can be rapidly and accurately made since almost the whole width of the groove is in the low power field of the binocular dissection scope. The strips may be used repeatedly by cleaning, autoclaving, and reparaffining.

Beadle, G.W. and Boris Ephrussi Collecting eggs and larvae. For collecting reasonably large numbers of eggs over known periods of time, 500 cc Berzelius beakers (without lip), stoppered with cotton enclosed in cheese-cloth, have been found convenient as containers for the adult flies. Eggs are collected on ordinary food heavily seeded with fresh yeast and held in boxes made of 26 gauge "Monel" metal. These are easily made from 2 by 3 inch pieces cut and folded to form a box 1-1/4 by 2-1/4 inches and 3/8 inches deep. A handle projecting 1 inch at one end is useful for handling these boxes with forceps. Young larvae can conveniently be removed at desired intervals from the food surface and be transferred to appropriate containers. Straight-sided finger bowls containing about 130 cc of food and closed with cheese-cloth held on with a wide rubber band, can be satisfactorily used for growing up to 100 larvae. The food surface should be heavily seeded with fresh yeast.

Crow, F. A. E. Egg Counts For counting daily egg output of single females a modification of the spoon technique is used. Small slices of cornmeal agar food cut from suitable blocks are placed on the spoons with a seeding of yeast. These have the advantages of being easily examined, readily detached and possessing a rough surface.

Mickey, George H. Collecting eggs. Our (Austin, Texas) routine method of getting eggs is to keep a single female and two or three males together for a few hours in a vial containing a spoon of food. About 4:30 or 5:00 P.M. the spoon is replaced with a fresh one in which the food has been poured and sprayed four or five hours earlier (in order to allow the yeast to start its growth) with a heavy suspension of fresh Fleishman's yeast containing a drop or two of glacial acetic acid. The food should be more fluid than that ordinarily used in food vials and is improved for the purpose of seeing the eggs by adding enough powdered lamp black to color it a dark gray. The flies lay better if they have not been etherized for at least 24 hours; they also lay more rapidly in the dark about 6 P.M. The vials should lie on their sides to give a horizontal laying surface.

The spoons are cardboard picnic spoons with the handles clipped off, the sides trimmed to fit the vials and the tips pointed in order to reduce the surface which may touch the bottom of the vial and crush the flies.

Schweitzer, Morton D. Collecting eggs. During the past year various techniques of collecting eggs have been tried. The following method has regularly yielded 100-600 eggs per culture per four hour period, with an average of 300. Not infrequently, on the first day of collection, the yield has been as high as 800-1300 in a four-hour egg-laying period. (D. melanogaster, pseudo-obscura, and to a small extent affinis and miranda)

The important precautions to be observed for optimum yield of eggs are:

(a) The females should not be etherized at any time prior to use for this purpose.
(b) The medium should be seeded with yeast at least 6 hours and not over 24 hours before use.
(c) The surface of the medium shoudl be slightly roughened just before being placed with the flies.

(d) The surface on which eggs are to be collected must be ventral to the flies.

The details of the procedure I have followed are as follows: Young flies, not over 24 hours old, are transferred to fresh food without etherization (20-40 ♀♀ and ♂♂). Two or three days later they are transferred to fresh food. At this time the medium on which the eggs are to be collected is prepared. It consists of ordinary cornmeal-molasses- agar with lampblack added to give contrast to the white eggs. The cornmeal is sifted before cooking. The food mixture is poured onto the ordinary type of paper milk bottle caps, leaving a margin of 1 cm. all around. When cool, the surface is uniformly seeded with fresh yeast. (Caps for 24 hours are prepared at one time.) The next morning the surface of the food on the caps is scraped with a metal tissue lifter. The flies are transferred to empty half-pint bottles which are capped with the prepared paper caps. The bottles stand with the caps down. New caps are substituted at appropriate intervals.

Eggs have been collected by this method continuously for a week or more at intervals of 2, 4, 6, 8, 12 hours. If the rate of oviposition falls off after a few days it may sometimes be renewed by transferring the flies to regular food bottles for 2-3 days. Strains that do not reach their optimum rate of egg-laying as early as the fourth day may be kept on regular food longer before beginning the experiment. (D. pseudo-obscura does well after 7-10 days from hatching, affinis and miranda even later.

If properly fitting caps are used (diam. = 1.625" for Bridges-type bottle, and 1.640" for most others), they may be washed and reused indefinitely. (Copied from DIS-4:65-66)

Schweitzer, Morton D. Handling eggs and larvae. When eggs are collected in the manner outlined above the usual high mortality due to handling and yeast overgrowth may be minimized by several precautions. After counting, the entire slab of food (or a segment containing an appropriate number of eggs) may be transferred to the surface of regular unyeasted food. If the surface of the food on the cap is sliced off with a scalpel just before use, the danger of yeast overgrowth is much reduced. An alternative method of transfer, that has given high percentages of imagines, is to allow the eggs to hatch on the food while it is still attached to the cap. The young larvae are transferred with a fine scalpel. In transferring larvae, an efficient method is to gently touch the scalpel to a larva, them touch the larva to a second one, etc. until 25-75 are adhering to each other. In this way the larvae are subject to a minimum of direct handling. (Copied from DIS-4: 66-67)

Hoover, Margaret E. Eggs for larval observations. In studies involving the embryological stages of Drosophila, the use of a synthetic medium may be found useful; especially if it is necessary to watch growing larvae day by day, a transparent food becomes essential. We have found the following technique satisfactory for egg-larvae counts.

EGGs collected from spoons containing a level amount of cornmeal-agar food blackened by finely powdered charcoal and painted with yeast are placed in a row upon the surface of Pearl's synthetic medium in a watchglass. If each individual is to be accounted for throughout larval life, 10-12 eggs in each watchglass will probably be found a sufficient number. The eggs are easily transferred by needle to the watchglass without injury. Enough yeast is carried along in the transfer so that the addition of yeast is unnecessary. Each day every watchglass can be inspected as often as desired and each larva can be accounted for. We have most satisfactorily used a Greenough binocular with 9 X oculars, 2.3 X objectives, 150 Watt light placed horizontally to the mirror so that the reflected light passes up through the glass stage and through the transparent food giving intense illumination.

If it is necessary to study the individuals further, the pupae can be transferred from the watchglasses to regular food vials where pupation will occur normally and the adult flies can be collected in routine fashion.

Danner, Edwin C. Methods for obtaining Drosophila eggs for embryological study.

In obtaining Drosophila eggs for fixation and sectioning or for study in vitro, the homopathic vials (3/4 X 3-1/2 inch) were found to give good results. (Shell vials of similar size may be better.) Approximately one half inch of banana agar medium is placed in each sterilized vial and allowed to solidify. Immediately upon solidifying, a strip of toweling (1 X 4 inch) with one end cut rounded to fit the vial is inserted so that the rounded end lies upon the medium and at right angles to the remainder of the strip which is pressed against the side of the vial. Moisture from the medium is absorbed by the toweling. Powdered yeast is then sprinkled upon the moist toweling and allowed to stand twelve to twenty-four hours.

In preliminary experiments, single pair matings resulted in good egg production. Virgin females approximately four and a half to five days old, when mated, usually laid eggs shortly after being fertilized. The eggs were laid on the growth of yeast on the strips of toweling. The parents may easily be shaken from the vial and the strip of toweling with the eggs upon it easily removed. Upon the removal of the eggs, the strip may be reinserted and the parents returned to the vial for further egg laying.

In collectin the eggs for study, the moist toweling was made to adhere to a piece of cardboard or filing card to facilitate handling. With the aid of a binocular microscope, the eggs were easily removed with a needle and were free from medium.

To remove the opaque chorion, the eggs were placed in a drop of distilled water upon a glass slide. The excess water was drained off and the slide placed under the direct light from a desk lamp with a fifty watt bulb to evaporate the remaining water and cause the eggs to adhere to the slide. This slight drying aids in the removal of the chorion with a sharp needle, the egg itself not shrinking from loss of moisture unless allowed to dry too long. For dissection purposes a #40 objective and #10 X oculars were used on a Spencer binocular microscope.

Altenburg, Edgar Eggs for ultra-violet treatment. Get eggs 3 to 3-1/2 hours after flies have been placed in bottle for egg laying. Keep them while laying in a dark place at about 26° C. and see that females are good layers, viz. young and fat, and that they are kept at about 25° C. for a day or two previous to laying. The flies should be removed from bottle in which they were placed for egg laying 1 hour and 10 minutes after they were put in the bottle. The eggs should be kept at 24° C. With a sharp razor blade cut a sharp edge on a blotter (preferably blue), the cut surface being at an angle of about 45° to the surface of the blotter. Moisten the blotter, and slide the eggs with the aid of a blunt needle to the edge of the blotter, so that only the polar cap projects beyond the cut edge. Treat so that rays strike polar cup only, rest of the egg being shielded by blotter. About thirty eggs can be arranged along the edge of one blotter and treated.

Gottschewski, G. Collecting eggs from weak stocks. Aus Stämmen, die im Hinblick auf Sterblichkeit und Legefreudigkeit durch Pärchenkultur über mehrere Generationen selektioniert sind, werden X 10 frischgeschlüpfte Drosophilapärchen ausgesucht, die drei Tage in gewöhnlichen Kulturgläsern beisammen bleiben. Die 3-4 Tage alten Fliegen werden dann ohne Ätherbetäubung in einen einseitig geschlossenen Glaszylinder (12,5 x 6 cm) gebracht, den auf der unteren Seite ein Deckel einer Petrischale abschliesst. Auf dieser Schale ist Futter, das durch schwarzes Fliesspapier verdeckt ist. Das Fliesspapier wird mit 3% igem Eisessig und ausgequetschtem Futtersaft stark angefeuchtet. Die Schale wird jede Stunde durch eine neue ersetzt; das Gelege ausgezählt und nach ca. 19 Stunden beginnt die Hauptmasse der Larven zu schlüpfen. Die Daten gelten fur 25° C. Durch einstündiges Ablesen der Larven erhalten wir 0-1 stündige Larvengelege. Bei guter Behandlung - beim Ablosen feuchter dunner Haarpinsel, gleichmässige Temperatur usw. - schlüpfen aus dem Eigelege 85-90% der Larven. Ich verwende gleichzeitig 6-8 Cylinder und erhalte durchschnittlich 2-300 gleichaltrige Larven, deren Variationsbreite bei dieser Methode auf das geringstmögliche Mass eingeengt ist.

Clancy, C. W. Two methods of ligating Drosophila larvae.

Larvae of Drosophila may be ligated with fine, human hair at any desired segment by placing two surgical-dressing forceps on a table under a wide field binocular microscope in such a manner that the jaws support between them a previously prepared loop of hair. The loop, into which the etherized larva is placed by means of a small camel's hair brush moistened with saline solution, lies in the center of the binocular field dipping into a small drop of saline lying on a thick glass slide. If the larva has been properly etherized, i.e., quickly and sufficiently, it will relax and extend itself the moment the saline solution touches it. Orientation with respect to the segment at which ligature is desired is made with the brush or by moving the forceps holding the loop. The surface tension of the drop of water tends to hold the larva and aids in placing the loop.

In case the exact position of the ligature need not be determined at the time of tying, an even simpler and more rapid procedure may be carried out that eliminates both the binocular and the forceps. A heavy glass rod (1.0 cm. in diameter and 25.0 cm. long) is clamped to a ring stand by one end so that the opposite or free end extends horizontally toward the hands of the operator can rest comfortably on the table and at the same time hold the loop of hair at the same level as the rod. The larva, etherized, relaxed, and extended, is first placed in a small drop of saline on the upper surface of the rod. Surface tension of the water holds the larva and enables one to slip the loop around it and tighten the knot in the desired position.

In connection with a preliminary study of pupation, several hundred larvae have been tied by the above two methods. When tied shortly before pupation the larvae survive and go through preliminary pupation changes.

Ephrussi, Boris and G. W. Beadle A technic of transplantation for Drosophila anlagen.

A technic of transplantation in Drosophila has been elaborated. By means of this technic imaginal discs of eyes, legs, wings and ovaries can be successfully implanted into larvae shortly before pupation.

The actual technic consists of the injection of the desired organ into the body cavity of a larva by means of a glass micro-pipette connected with the capillary tube and syringe of the standard Chambers' micro-manipulator. The pipette is made in drawing out with a micro-burner a glass capillary with an external diameter of about 0.7 mm. and a wall thickness of about 0.1 mm. to a finer capillary shaft of an external diameter from 0.1 to 0.16 mm. The bore of the shaft should be from 0.06 to 0.12 mm. The length of the shaft should be about 2-3 mm. At its base a constriction is made in the bore by heating with a horizontal micro-flame. The function of this constriction is to block the tissue in the shaft, thus preventing its entering the larger part of the pipette, and also to act as resistance to the flow of liquid through the pipette. The pipette should have a very sharp point, which can be obtained by grinding on a fine-grained hone.

In our experiments the dissection of the donor-larva is made free-hand in a drop of 0.7% physiological salt solution. The host larvae are anesthetized on a glass slide in a simple glass vessel. After they are etherized and extended, they are moistened with a drip of Ringer, which tends to produce more nearly complete estension. They are then dried with filter paper and after a few minutes adhere to the slide.

The injection is made free-hand under a binocular microscope. Our equipment consists of two binoculars arranged so that they can be used from opposite sides of a narrow table. Two persons cooperate in the operation: one holding the larvae with a blunt curved metal needle, the other making the actual injection. (The complete description of the technic will appear in the American Naturalist.)

Beadle, G. W. Pigmentation of Malpighian tubes in larvae of D. melanogaster.

During the course of work on transplantation, it has been noticed that the Malpighian tubes of larvae of certain eye-color mutants are pale yellow or practically colorless, while those of wild-type larvae are distinctly yellow. Thus, ca, car, cm, g^2, lt, pP, rb, and w larvae appear to have very little or no pigment in the tubes. It is clear that these, and probably many more mutants, can be classified in the larval stage. To do this, it is not ordinarily necessary to dissect the larvae; the color can be seen through the body wall. The ability to identify particular mutant types in the larval stages obviously can be used to advantage in cytological studies, transplantation, etc.

Hoover, Margaret E. Some uses of Beadle's Malpighian tubes technique.

Although we have worked in a preliminary way for only a short time with Beadle's suggested technique utilizing the colors of the Malpighian tubes, it seems to offer interesting and important possibilities. The difference between yellow and white tubes can be easily seen in three-day old larvae and with practice the distinction can be made in younger ones. Careful examination with good strong lighting is necessary but the difference can be seen with accuracy. Such a technique can be put to good use in cases where an X-chromosome carrying a deficiency is balanced against dl-49 carrying garnet eye color. If ♂ carrying the deficiency die during larval life, the exact extent of survival can be accurately determined by isolating and observing the ♂ larvae with yellow Malpighian tubes. Moreover, by first observing the Malpighian tubes of the ♀ larvae used in making salivary gland chromosome preparations of such deficiencies, the use of ♀♀ not carrying the deficiency can be avoided.

Whittinghill, Maurice An aid in arranging flies for separating or counting them.

To facilitate sorting and counting flies it is advantageous to have them arranged approximately in a line, which may be easily formed as follows. The flies are emptied from the etherizing bottle upon a card which has along its middle a flexible fold by which the card may be bent to form a trough. A few strokes of the brush distribute the pile of flies evenly along this groove. The card is now held flat with one hand while the other goes down the line casting each fly quickly to one side or the other with a brush handle or needle, thus sorting into two groups. If further classification is necessary, these two groups may be swept to opposite ends of the card, or to different cards, and each group easily arranged for the next sorting. After such a sorting each of the groups is found to lie in approximately linear order, which makes counting simple. Finally the card may be bent again to make a chute for dispensing the flies accurately into the morgue.

Stern, Curt Technique for the study of certain genetic constitutions in hypodermis spots.

One tool in studies on gene action is the production of mosaic spots. Their appearance is based primarily on the occurrence of somatic segregation. A heterozygous cell Aa segregates into two cells AA and aa. If aa represents the genetic constitution to be tested and if the cell aa is viable and divides, a spot will appear. Often the genetic constitution to be tested in spots leads to inviability of the segregate thus not resulting in a visible aberrant area. The frequency of somatic segregation is rather variable so that conclusions as to inviability of certain constitutions can be drawn with reservation only, considering the possibility of absence of segregation. However, the following method furnishes reliable controls. Let a be the gene to be tested and b and c genes in the same linkage group effecting hypodermal characteristics in small spots. By mating flies of the constitution abC/ABc are produced. Somatic segregation will lead to abC/abC and ABc/ABc cells. The latter, known to be viable, will be able to produce a spot; the former, if viable will appear in direct contact with the ABc/ABc spot as an abC/abC twin spot. If aa leads to inviability no twin spot will be formed. In case of sex-linked genes yellow (y) may be used for b and singed^3 (sn^3) for c, so that either only single sn^3 spots of y next to sn^3 twin spots will be found (both y and sn^3 being recognizable as characteristics of even-single setae). Somatic segregation is caused by four strand crossing-over. If it occurs to the right of all loci studied, the foregoing holds true completely except for developmental reasons which may make the mosaic areas so small as not to cover at least one seta per single spot. Somatic crossing-over between the genes studied will not lead to twin spots. However, the frequency of somatic crossing-over at the spindle fibre region is high enough to produce a sufficient number of potential twin spots.

Frequency of spots: Variable in different experiments from one spot on fractions of a percent to one spot on ten and more percent of all individuals. The frequency is increased considerably if the individuals carry an autosomal Minute (use stocks like Rochester #68, DIS-5)

Size of spots: From one seta to whole imaginal disk, very rarely larger.

Location of spots: Variable. The smaller spots, which in most experiments are the frequent ones, occur preferably on the abdominal tergites. Careful inspection under about 30x magnification is necessary for detection. (See note in DIS-5 on "foot-focusing device").

Harnly, Morris H. Wing measurements. The following method has been found satisfactory for making wing measurements: The Spencer Drawing Apparatus No. 345 MS (list price $62.00) on which a compound microscope can be mounted is used to project the wings. A 16 mm. objective and 10 X ocular are used. The size of the projected wing is determined by the distance of the microscope above the drawing board. When first setting up the apparatus it is advisable to project a wing and determine a height that will place the entire wild-type wing on the drawing paper. Ordinary 8 x 11 paper can be used for the drawings.

Having established the proper height of the microscope above the drawing board, a ruled 2 mm. slide is placed on the microscope stage and projected. This distance of 2 mm. can be marked off on a straight line on a permanent record sheet. Thereafter, whenever the apparatus is set up exactly the same magnification can be obtained by a proper adjustment of the height of the microscope above the drawing board (a slight movement of the draw tube may aid in this) using the 2 mm. slide and the record sheet as checks. The wing is removed from the fly with a McClure's angular-or-flat Iridectomy Scissors #c991 figure 2 (list price $9.00, Standard Scientific Supply Co.), mounted in 95% alcohol, projected and drawn. The length can be determined directly by projecting the 2 mm. ruled slide onto the drawings. An area equivalent to 4 sq. mm. can be obtained at the same magnification by projecting the ruled 2 mm. slide and measuring the square drawn with a Keuffel and Esser Compensating Polar Planimeter No. 4242. This will give by division the value in sq. mm. of one unit on the vernier. Measurements with the planimeter of the area of the wing drawings can then be converted into sq. mm.

Timoféeff-Ressovsky, N.W. and K.G. Zimmer. On the technique of radiation-genetic experiments. From both the genetic and physical points of view we want to lay stress on the following rules, the observation of which will be of great help for comparing and analysing the results of radiation-genetic experiments in Drosophila species obtained by different authors.

1. The biological genetic conditions and methods of the experiment must be taken into consideration, and, if specifically deviating from the generally known and used standard (in respect to age and stage of irradiated flies, developmental stage of germ-cells at the time of treatment, methods and accuracy of detection of mutations, etc.), should be mentioned.

2. In experiments upon mutation rates of single genes uniform and selected material should be used.

3. When using X-rays, ionometric measurements of the tube output and its steadiness ought to be carried out before irradiating the flies. The conditions of measurement and irradiation must be exactly the same in respect to tube current, voltage, presence or absence of scattering substances in the neighborhood, and distance.

4. It is preferable to use the international r-units of X-radiation instead of other units not generally adopted.

5. It is necessary to state, besides the dosage applied, also: a) the time and intensity of irradiation (i.e. whole time of exposure, r/min, and whether fractioned or not), b) kilovoltage, c) filtration, and, if possible, d)halve-value-layer of the X-ray beam. For very soft X-rays (so-called Grenzrays) cellophane is the best material for halve-value-layer determinations; for all other qualities of X- and gamma radiations copper ought to be used rather than aluminium.

6. For soft radiations absorption within the flies becomes very important, and proper allowance for dosage reduction in deeper layers of the flies has to be made; this point, which can easily introduce errors as great as 100 per cent, has frequently been overlooked in the past. Thus, in the case of soft rays, beta- and cathode- radiations, the absorption in fly-tissues must be measured, and the mean depth of gonads during irradiation must be determined.

7. When planning experiments under special conditions (e.g. with extra soft or extra hard radiation, very high or very low intensities, in combination with other physical or chemical factors or stimuli, etc.) advice of a competent physicist, preferably one who is interested in such problems, ought to be asked for and followed.

8. Experiments with gamma-rays from radium require considerable amounts of radium or Radon. Such work is, therefore, rather dangerous if special measures of protection are not arranged and followed. During the last few years it has been shown that gamma-rays can be measured in r-units ionometrically. It is of course desirable to use these units in the future. If exact determination of the dosage output in r-units of the radium applicator used cannot be carried out, the following must be stated: a) the number of mgrs. of radium element, b) filtration (thickness and material), c) time of exposure. and d) distance. The radium (in needles, tubes, or other form) must be so arranged in the applicator as to give a large enough homogeneous field of gamma-rays. All details of the experimental set up and of the radium applicator (preferably with

diagrams giving all lengths, distances, etc. in mm's) have to be described.

9. During irradiation the flies ought to be kept in a container of light-atomic material, such as cellophane, gelatine, or bakelite, which has a wall-thickness equivalent to that of the ionisation chamber of the dosimeter.

Dobzhansky, Th. Collecting, transporting, and shipping wild species of Drosophila.

Usual Drosophila culture bottles can serve as traps. A sturdy string is tied around the bottle neck to facilitate its hanging in a convenient position on branches of trees, bushes etc. A layer of bait a few centimeters thick is placed on the bottom of the bottle, and covered with a piece of a paper towel or filter paper to absorb excess moisture. Fermenting banana mush is most satisfactory as bait. Ripe bananas are mashed with the aid of a spoon or a fork; some drops of fresh yeast solution is added, and the bottle is left standing for about 24 hours before use (if dry yeast is used this time is considerably lengthened). The bait remains good for at least four or five days after first used. The traps are exposed in such a way as to be readily accessible, and left undisturbed for a few hours; no useful purpose whatever is accomplished by leaving them exposed for days. In the case of some species, notably pseudoobscura and its relatives, it is important to expose the traps in the late afternoon, since these flies do not come into traps on hot days before sunset. On cloudy days they may be caught any time. The above bait attracts quite a number of species of Drosophila, including some, though probably not all, feeding on fungi. When a sufficient number of flies are in a trap, it is closed by the usual cotton stopper, and transported to camp (if properly packed such bottles can be transported for miles even on pack saddles without harm to the flies). For further transportation and shipping the flies should be placed in shell vials containing solid banana-agar food. Banana agar is prepared in the laboratory, poured into the vials, sterilized in an autoclave under pressure, and cooled in a slanting position. The openings of the vials are stoppered with sterilized cotton and wrapped in wax paper to prevent excess evaporation. Vials so prepared can be then carried for a month before use. Before the flies are put in them, a drop of yeast suspension is placed on the food, and a piece of filter paper inserted. Then the flies are transferred from the traps to the vials (a glass funnel of a proper size facilitates this operation greatly). Some wild species are exceedingly sensitive to heat. If they have to be carried around for any length of time in hot weather, vials with the flies are placed between layers of cotton in a metallic box, and this box is placed into a larger one, the space between the two being filled with cracked ice; changing the ice in this improvised ice box once a day is sufficient in any summer heat. If no ice is obtainable, the box with the flies should be wrapped in a moist towel and exposed to the wind. It is desirable to ship the material collected to the laboratory as soon as possible. During the summer heat shipment via air mail has to be resorted to despite the expense involved. For this purpose the vials with flies are placed in cardboard boxes with cotton and wrapped like any other parcel. In some states the importation of living insects is prohibited, so that special permits may be necessary.

Cytological technique

Painter, T.S. Aceto-carmine technique for salivary chromosomes. The writer has worked almost exclusively with temporary aceto-carmine mounts and the commments given below apply only to that type of preparation.

Much difficulty has been experienced in getting a good iron aceto-carmine stain. Powdered carmines, from many different sources, have been tried including the certified product of Coleman and Bell, Grübler's Karmin Rubrum Opticum, and Carmine 40 from several sources. The trouble has been either that the stain would not take or was not selective enough or else it would not keep, at ordinary room temperature here in Texas for more then a few days without going bad. At present we are using some uncertified carmine manufactured by Coleman and Bell and are obtaining a satisfactory stain in the following way. In one flask an excess of carmine is simmered, under a reflex condenser, in 40 per cent acetic acid, for an hour or two, when it is cooled and filtered. In another flask carmine is boiled in 60 per cent acetic acid similarly, and after cooling is filtered. A trace of ferric acetate is added to both stock solutions. From time to time, as needed, the two stains are mixed in equal proportions. We are unable to explain why this procedure gives a good stain but it works.

The salivary glands are dissected out in Ringer's solution (cold-blooded or frog formula) and transferred to a clean slide with a pipette. The Ringer's is quickly removed with a pipette and iron-aceto-carmine is flowed over the glands from one side. After a few moments the first stain is removed and fresh stain is added in considerable excess. The slide is put to one side and allowed to stand until a little of the carmine begins to precipitate around the edge, a matter of 15 to 25 minutes depending on atmospheric conditions. A cover slip is now placed over the glands, and the excess stain removed with a pipette and filter paper. Being careful not to let the coverslip move, the preparation is next blotted with a good deal of pressure, a process which usually frees the individual nuclei from the surrounding cytoplasm. Under a dissecting binocular, the individual nuclei are crushed with a blunt needle, by the pressure applied locally to the cover slip, and then after blotting the slide once more to remove all traces of the stain the coverslip is sealed with vaseline or melted paraffin.

The type of light filter used for the examination of preparations is the BG 7 optical glass filter put out by Zeiss.

Marshak, A. A rapid method for making permanent mounts of Drosophila salivary gland chromosomes. (1) A saturated solution of aceto-carmine is prepared by boiling carmine in a 45 per cent aqueous solution of glacial acetic acid for several hours. A reflux condenser is attached to the flask containing the solution in order to prevent changes in concentration by evaporation. A clear dark red solution is obtained, either by sedimentation or filtration. No iron is added at any time.

(2) The glands are dissected out and stained in deep depression slides for not longer than fifteen to twenty minutes. If left for a longer period the chromosomes become fragile and cannot be well stretched.

(3) The glands are mounted on a microscope slide and washed with fresh aceto-carmine to remove any debris that may be present. They are then covered with a square coverslip and the excess fluid taken up with blotting paper.

(4) The chromosomes are then spread by pressing on the coverslip with a dissecting needly. The coverslip must not be allowed to slide or the nuclei will be rolled into dense useless masses. This is easily prevented by pressing firmly on one corner of the coverslip with the finger. This step is executed under a dissecting microscope so that each nucleus may be observed and adequately spread.

(5) A saturated solution of carmine in glycerine is then put around the coverslip and a piece of absorbent paper placed against one edge of the coverslip. The slide is then put away until the glycerine has displaced all the aceto-carmine under the coverslip, usually over night. The glycero-carmine solution is prepared by dissolving the carmine in warm glycerine and then filtering. If ordinary glycerine is used in this step, the preparation will fade after a few days.

(6) The slide is immersed in alcohol to remove the excess glycerine and then blotted. It is then sealed with balsam, gum mastic-paraffin or any other suitable seal.

By this technique it has been possible to mount three hundred glands in two days and have them all preserved in excellent condition for observation. After one month there has been no noticeable change in the chromosomes. Furthermore, it is possible by this technique to stretch the chromosomes a great deal without breaking them.

If it is desired to mount the glands in balsam, the slides may be allowed to stand with the glycerine for a day or two longer. The coverslip can then be readily slipped off, or pried off with a fine needle, or floated off in absolute alcohol. It is then cleared in clove oil and xylol and mounted in balsam. The loss of material by this technique is much less than with the method of removing the coverslip in aceto-carmine.

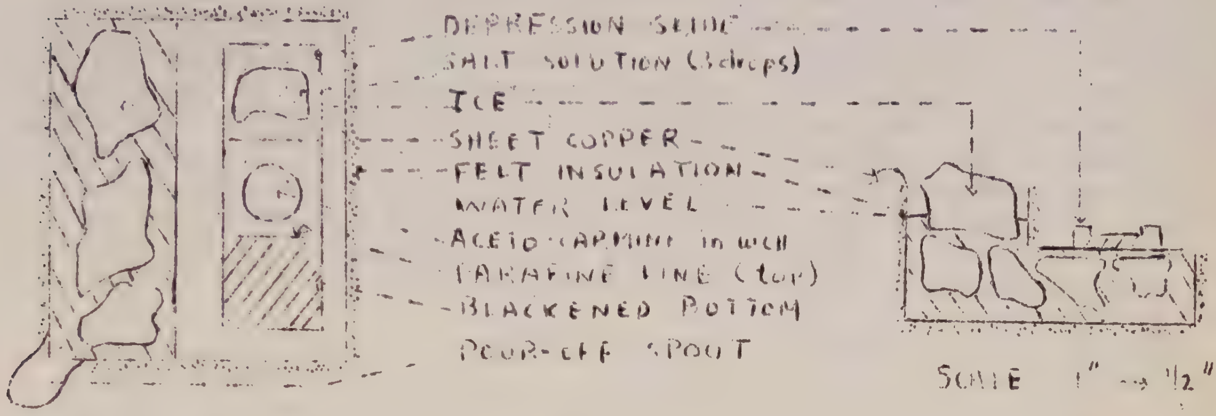
Bridges, Calvin B. Current method for permanent aceto-carmine smears. Trial of various modifications of the methods for making temporary and then permanent preparations of smeared cells from salivary glands and other tissues have been carried out by various workers here. The method is so uniformly reliable and yields permanents of such high quality that it is no longer customary to carry out even preliminary examinations on temporary mounts. Permanents are essential for continual rechecking of the banding in each case as new information or material raises questions.

A prime condition of success is that the larvae be uniformly and thoroughly overfed with yeast and raised at a low temperature (see notes by J. Schultz). Fully adult but still unpupated larvae should be used, since degeneration of the cells starts immediately with pupation. Female larvae should be selected and males discarded, since in males the chromosomes, especially the X, are paler in stain and less turgid than in females. Best are females with one or two extra Y-chromosomes, where the extra "inert" material leads to plump easily-stained chromosomes. Attached-XY females are used as the source of an extra Y whenever it is otherwise desirable to make an outcross of a balanced autosomal stock (to get heterozygotes freed from the balancer). The giant attached-X females, formerly recommended, are no longer used in preference to other attached-X females, since the extra large chromosomes found in giant females are too resistant and elastic to stretch well in the smearing. Female larvae can be distinguished from males by their tiny colorless transparent spherical ovaries attached to the yellowish opaque lateral fat-bodies between the third and fourth branches of the trachae, counting from the rear. Male larvae have large ellipsoidal testes each embedded in the fat body.

Dissection and fixation of the salivary glands at near-zero temperatures seems to give better detail and less capsulation of the banding (see notes by Schultz). For piercing the larvae and other materials, a large low-sided basin or tray is filled with cracked ice. In the ice are bedded salt cellars (black) for prechilling the larvae, bottles of physiological salt solution and of concentrated aceto-carmine and special depression slides for the dissection and fixing. The depressing slide (see figure below) is 1" x 3" x 1/4", with a 5/8" circular well, with vertical walls, 1/8" deep. The upper surface of the slide is finely ground. The end regions are marked off for dissecting spaces by lines drawn in melted paraffin to keep the salt solution from running into the well of the slide. The bottom of the slide, under these end regions, not under the well, is painted black for contrast to the larvae.

The larvae are put in the dry depressions of the chilled saltcellars for about five minutes before dissection. Because of the chilling they stop crawling about and are much easier to handle and dissect. For keeping the larvae and the liquids chilled during dissection and the first stages of fixing, a special insulated iced platform (see figure below) can be made of soldered sheet copper with felt covering, of a size to fit conveniently on the stage of a dissecting microscope.

The interior of the dissecting platform is filled with ice at the time the tray is filled. The platform is put on the dissecting microscope stage so that the light comes from the far side along the ledge. The well is filled nearly level full with the chilled aceto-carmine. (Aceto-carmine can be made by boiling for an hour 45% acetic acid with an excess of carmine powder (Coleman and Bell is good) in a flask with reflux condenser, and decanting for use the clear liquid after cooling and thorough settling). On the ground glass surface at the far end of the slide are put three drops of salt solution for the dissecting bath.



In dissecting (at 15 diameters magnification) cut off anterior end of larva, as close behind mouth hooks as possible, by pressure with side of needle tip against non-slip ground glass surface, while holding larva with another needle. From cut-open end emerge first the pair of salivary glands and then other organs. The glands (transparent club-shaped bodies attached to each other by the short branched duct which led to the base of the mouth hooks) are cleared from the fat bodies and picked up together on the tip of a needle and immersed under the surface of the aceto-carmine in the well.

In case the glands break apart they can be made to stick together on the needle tip, turning this upside down and then immersing under the aceto-carmine for 30 seconds before scraping them off. Up to about 16 pairs of glands may be fixed together in one well. Avoid excessive iron (from the needles) in the early stages of fixation. After the required number are in the well, the slide may be set aside at room temperature or higher for continuing the fixation at a faster rate. Short fixations before the smearing are liable to result in pale chromosomes and distortion and tearing through adhesions of the still soft material of the strands. Fixation from 1 to 2 hours seems best. Longer than about 4 hours may lead to fragmentation of the chromosomes upon smearing. With long fixation refill the well as the acetic acid evaporates.

To make mounts, put albuminized slide (see notes of Baur) on white plate on stage of dissecting scope. Put 3 drops concentrated aceto-carmine on slide and spread evenly over albumin surface. Four pairs of glands may be put in a row on the slide and covered with a 22 x 40 mm. cover. Slides should not be over 1.20 mm. thick and covers not over .16 mm. Avoid bubles in lowering cover in place; The albuminized slides must have been protected against dust and lint while drying and during storage and the covers carefully cleaned, otherwise any particle would prevent the cells being crushed properly.

Place slide, with cover glass well centered, on lower half of folded paper towel and drop upper half over the cover. Gentle pressure on top half, without side slip, removes excess of aceto-carmine, leaving only faint tinge of pink on slide and the glands partly spread and crushed. Complete the spreading and crushing, with slide on white plate on stage, by vigorous sweeps with side of needle tip, held nearly parallel to surface, holding corners of cover with fingers to avoid all side slippage. If too much fluid has been left in slide the spread chromosomes will later change their place rather than stick where they are spread. The thin film of albumin makes a soft bed which saves the chromosomes from being crushed, as would easily occur between bare slide and cover glass, and causes practically 100% adherence to the slide.

The fresh preparations are now "ripened" by storage in saturated 95% alcohol vapor over night. A large glass vessel 6" deep is lined on bottom and sides with several layers of filter or towel paper, 95% alcohol poured in until the paper is melted and a little excess liquid is left over on the bottom. The slides are stood on end around the sides and the whole covered tightly. This vapor method of replacement of aceto-carmine (or other liquid under a cover glass) by alcohol gives a slow and perfect dehydration with a minimum of shrinkage. Vapor condenses on the upper end of the slide and the displaced liquid seeps off at the bottom.

After ripening and dehydration (the material also sticks to slide better after ripening in alcohol vapor) the slides are immersed in 95% alcohol, where they may be left indefinitely. The covers may detach spontaneously; but they can be easily removed by placing the side in a shallow Petrie dish (J.C. Li), covering with 95% alcohol and holding 2 rear corners of cover (against slippage) with fingers, while a flat thin point of bent needle is slipped like a wedge under opposite edge. The slide is left in the alcohol while the cover is cleaned of traces of tissue. The slide is drained briefly, laid on its back and 2 drops of Euparal (thinned slightly with thinner or with 100% alcohol) dropped on the glands. The cover is put on swiftly (to avoid drying and also condensation of moisture) and air bubbles avoided. The excess of thin Euparal is squeezed out by folded paper which absorbs it as it emerges (avoid slippage while pressing). After drying of slide, clean off excess of Euparal with cloth barely moistened in 95% alcohol. Slides continue to clear and improve, and are fully equal to balsam slides, without the time and expense and shrinkage involved in treatments with absolute and Xylol.

Schultz, J. Notes on methods for salivary chromosomes. Larvae used for salivary gland preparations should optimally be from single females which are transferred to fresh 1/2 pint culture-bottles daily. Several such transferred pairs provide the required numbers of uniformly well-developed larvae. The larvae should be raised in a cool place, preferably a 19° cold room, and the culture medium should be enriched with yeast (sterilized) or extra live yeast (1/8 -1/16 cake of Fleishmans yeast) sprinkled on the food surface when the larvae are half grown. Absorbent paper should be provided for the adult larvae to emerge upon, and the easiest way to collect larvae is to pull out this paper and pick off the fully grown individuals.

Glands dissected at practically zero temperature and with the fixation started at that temperature seem to give sharper detail and less "capsulation" of the banding than material dissected and fixed at room temperature. The Ringers solution (or 0,73% Na Cl solution) and the aceto-carmine should first be chilled to near zero by standing on ice. The larvae also should have an ice treatment of five or more minutes before dissection. The dissection and first stages of fixation should be carried out on a depression slide held at low temperature (for details of procedure see notes by Bridges). After the fixation has proceeded for five or more minutes at the low temperature, the remainder of the fixation can be carried out at either room temperature or higher. With higher temperatures less time is needed for the fixation and staining to reach a suitable stage.

Mounting in Euparal, directly from 95% alcohol, has been found perfectly satisfactory if done rapidly in a dry atmosphere.

Bauer, Hans   Notes on permanent preparations of salivary gland chromosomes.

Perfect attachment of smeared cells and chromosomes to the mounting slide can be obtained by use of a film of albumen on the slide. The albumen solution is made by mixing togehter 100 cc distilled water, 25 gm powdered egg albumen (Merck) and 0.5 gm thymol. After the mixture has stood several days and the undissolved albumen has settled, the top clear portion is decanted for use. A drop of albumen solution is spread evenly and thinly over the whole slide by scraping with the end of a second slide whose edge should be unchipped. The albumen film must be thoroughly dry before use! For use, the dried albuminized slide is put on a level place and three drops of aceto-carmine spread evely over its entire surface. The stained glands are placed in this fluid film, covered with a cover glass (air bubbles must be avoided) and crushed by pressure.

The gradual replacement of the aceto-carmine by 95% alcohol can be made by the vapor method of Bridges (see above) or by putting the slide in a staining jar filled with alcohol only high enough to cover 1 or 2 mm of the lower edge of the cover glass. After half an hour the slides are ready to be transferred to another jar filled completely with 95% alcohol. Here the cover slip usually detaches itself after a short time; otherwise it can be removed by needles. Mounting is done according to the methods described above by Bridges.

In cases of too strong stainability of the cytoplasm by aceto-carmine, the Feulgen method is advisable (Feulgen, R.,

1926, Handbuch der biol. Arbeitsmethoden 5: 2,2 Hälfte. - Bauer, H., 1932, Zeits.f.Zellf.u.mikr.Anat. 15. - The receipts are to found in the last edition of Lee's Vademecum.)
Slides are prepared by the aceto-carmine method to the stage of detached cover glasses; or slides already mounted in euparal can be soaked in 95% alcohol till the cover glasses detach. The slides are then transferred through 70% alcohol and distilled water into chrom-formol for postfixation (3 parts of 1% chromic acid and 1 part of formalin); duration 10-20 hours (overnight). Thereafter the slides are washed for 10 minutes in running water and, after transfer through distilled water and cold normal H Cl solution, hydrolyzed for 30-40 minutes in normal H Cl warmed to 60° C. on a water bath. After hydrolysis they are transferred through cold normal H Cl and distilled water into the fuchsin-sulphurous acid for 1-3 hours. After the staining the slides are washed in a large jar with tap water, moving them backward and forward several times (washing in SO_2-water is not necessary); the side of the slide carrying the objects should always be turned in the direction of the movement. Then they are transferred through distilled water and 70% alcohol into 95% alcohol. There they should remain until the red color in the albumen layer, caused by the decomposition of the fuchsin-sulphurous acid, is completely removed. Then the slides are mounted in euparal, or, through absolute alcohol and xylol, in Canada balsam. Only the chromatic structures (containing thymus nucleic acid) are stained; the staining is not quite as intense as that by aceto-carmine and does not seem any sharper or less diffuse in the demonstration of the banding. During the postfixation the chromosomes, due to shrinkage, become thinner.

These methods are applicable also to ganglia, ovaries and testes.

Lawrence, Elizabeth Gay (Carnegie Institution of Washington, Baltimore, Md.) Note on the use of dioxan in making permanent aceto-carmine preparations.

At the suggestion of Mr. C. H. Miller of this laboratory, dioxan has been tested as a medium to replace alcohol and xylol in making permanent smears. Only preliminary tests have been made so no detailed method has yet been worked out. The general procedure, however, seems simple and the preliminary results very satisfactory.

Cover slips are soaked off in dioxan, then the material is rinsed in dioxan for a few seconds and mounted directly in balsam. Dioxan is relatively inexpensive. It may be procured from the Carbide & Carbon Chemicals Corporation, Carbide & Carbon Building, 30 E. 42nd Street, New York City, and presumably from Grübler in Germany. Its use has been described in the following papers:

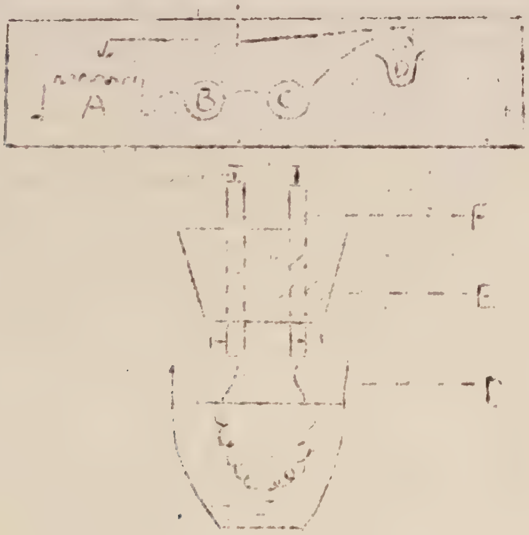
"The Use of Dioxan in the Embedding of Microscopic Objects," by Heinz Graupner and Arnold Weissberger, Zool. Instit. & Chem. Lab. of the Univ. of Leipzig; Zool. Anzeiger, Bd. 96:204-206,1931.

"The Use of Solutions in Dioxan as Fixatives for Frozen Sections", by Heinz Graupner and Arnold Weissberger; Zool.Anzeiger, Bd. 102:39-44, 4 abb., 1933.

Kaufmann, B. P. Technique for spreading salivary chromosomes. (1) In making aceto-carmine smears of ganglia and salivary glands we have secured more uniform distribution of cells and less breakage by flatening with a weighted roller rather than with a needle or similar instrument, where the results depend on the pressure exerted by the technician. For this purpose we use pieces of glass tubing, filled with about 150-200 grams of mercury, and securely corked. A horseshoe-shaped wire with the ends inserted in the corks may be used as a handle in drawing the roller across the cover.

Griffen, A. B. Sealing slides with paraffin. In the preparation of salivary chromosome slides for rapid checking of breaks and translocations, it is seldom desirable to spend long hours in making permanent mounts of all the preparations; it is far more practicable to make well-sealed temporary slides which may be made into permanent records as the worker sees fit. Paraffin, applied at smoking heat with a small brush, forms a neat, rigid and easily removable seal for such slides; after the initial use of the material the longitudinal paraffin strips may be flicked away easily from the cover-slip and the slide treated for permanency by Bridges' technique. For providing paraffin the apparatus described below is very handy.

A small 15 cc crucible is used as the melting pot; into this vessel, filled with paraffin, is suspended a crescent spiral of Chromel-A-22 resistance wire with a 22.5 ohm rheostat, a safety fuse, and a toggle switch in the circuit. After the initial adjustment of the rheostat a flip of the switch instantly produces smoking paraffin.



A - rheostat
B - fuse
C - switch
D - crucible
E - two-hole rubber stopper
F - attachment post

Bridges, Calvin B. The Examination of salivary chromosomes. The detail present in salivary chromosomes extends in fineness beyond the resolving power and definition of the best microscopes. Aside from the use of very expensive and cumbersome ultra-violet apparatus with photography, the greatest amount of this detail can be seen by the following procedure, according to my experience. For objective and ocular I prefer 90 X apochromatic 1.40 N.A. with 10 X compensating ocular. Second choice is 120 X apochromatic objective with 10 X or 7 X ocular, and third choice is 90 X 1.30 N.A. with 10 X ocular. Oculars higher than 10 X seem inferior in results, though 12.5 X offers possibilities. For substage condenser 1.40 N.A. achromatic is best, and any achromatic is better than any aplanatic or other type of condenser. It is as imperative that the condenser be used oil-immersed and focused as it is for the objective to be so used. A front surfaced aluminized mirror (vapor deposited) is definitely superior to other mirrors and is more flexible in its setting angles than is the total-reflecting prism, which is otherwise the best. For examinations a binocular body with inclined ocular tubes is to be preferred. For camera-lucida drawings a monocular straight tube should be substituted.

adjustment of light under all conditions of filters, outside

Too much attention cannot be paid to the illumination of the chromosomes. For critical examinations one can employ a Bausch & Lomb thirty-nine Dollar lamp (nearly as good as the "research" lamp). The light is from a 6-volt, 18-ampere ribbon-filament tungsten incandescent lamp, of projection type, with bayonet (not screw) base and brazed (not soldered) center contact. This lamp is run on alternated current transformed from 110 to 6 volts. Intensity of the light is controlled by a variable resistance, of not less than 175 ohms and not less than 1.5 amperes capacity, in the primary 110-volt circuit to the transformer. Use of this resistance is imperative for exact illumination and eye-fatigue. Also it increases the life of the lamp many times its rated life.

The light from the ribbon filament is brought to a sharp focus in air as near as practical to the front of the condensing lens. This is accomplished by turning the loop of the filament toward the back of the housing and moving the lamp mounting back in the housing and racking the condenser to its limit forward. After the filament is carefully aligned with the center of the condenser, an iris diaphragm is mounted in the plane of the image of the filament. The mounting support may be made as a right-angles wooden trough extending forward from the cylindrical lens casing below, and wired tightly to it. The iris is mounted to a smaller trough which slides in the support trough. To find the exact setting for the iris, put on the stage (condenser oiled) a slide and focus on it with the oil immersion objective. Next place the lamp with the field iris about 15 cm. from the mirror face and center the beam carefully on the mirror. With open field iris focus the edges of the ribbon filament sharply in the field by racking the condenser screw. Next close down the iris to about 2 mm. and slide it along its trough until its edge is prefectly sharp in focus. The iris carrier can thus be screwed permanently to its supporting trough. In critical use this field iris is closed to give a lighted area about half the diameter of the field of the objective, and its edge (as well as the ribbon at its midpoint) should be clearly in focus in the plane of the preparation. The iris beneath the condenser should then be closed down until the light haze in the marginal field narrows until it disappears by coinciding with the edge of the field iris image. This procedure does not use the full aperture to the condenser (approximately 0.6) but increases definition by contrast.

The carmine stain absorbs a maximum in the green at about 530. Hence to get greatest contrast a green filter should be used whose transmission is entirely within the absorption band of carmine. The best filter for maximum contrast is Wratten No. 62 (mercury Green). This is a dense filter and is recommended for cataloguing and being certain of the existence of the very faintest lines. For general use Wratten filter No. 61 (N) is the best, since it allows discrimination of the relative intensities of the lines through having a broader transmission (on both sides) than is the absorption band of carmine. Nearly as good as 61 for general use is 58A-B2 (dark). Filters 61 and 62 should both be obtained and none others are needed for aceto-carmine work. They should be mounted as close to the substage condenser as possible, to avoid fading of a spot where light is concentrated.

In checking details of banding search for places where the chromosome (instead of lying lax) is stretched to two or four times its normal length. The stretch comes almost exclusively *between bands* and the bands are thus moved apart sufficiently to be distinctly resolvable as separate entities. Many apparently single bands are thus seen as doublets and an astonishing number of faint lines are brought to view. Final checking of bands is made by oblique light, cast along the axis of the chromosome. The oblique light is best obtained by sliding a black card partly over (below) the intake face of the substage condenser and as close to it as possible.

Microscopical examinations should always be done in a brilliantly and uniformly lighted room, never in a darkened room, cubicle or dark corner. When the general outside illumination and the microscope field exactly match in intensity there is a minimum of eye fatigue and dazzle by sudden entry of light through an expanded (dark-adapted) iris! Spotty sidelights and highlights should be cut out by a semi-cylindrical shield, made of Bristol board and wood and mounted on the microscope body. The interior of this shield and the tops of the objective and draw tubes should be painted neutral gray of very light color and flat (non-gloss) tone, instead of the customary glossy black.

It is recommended that camera-lucida drawings be made at a standard magnification of 5,000 diameters, which is large enough to represent the detail seen. This exact magnification can be obtained by trial of projection of a ruled stage-micrometer upon the drawing surface and adjustment of the length of path of projection until 10u on the stage micrometer becomes exactly 50 mm on the drawing board. The microscope and lamp can be mounted together on a board and this board raised above the regular table by blocks to an amount exactly determined by trial. With 90 X objective and 30 X ocular and with mirror arm fully extended, the microscope base needs raising approximately 1-1/2 inches above the drawing level on the table.

Anthes, E.H. Critical illumination for microscopic research The need for a correctly designed illuminating unit for use with a research microscope is no less than the need for carefully constructed optics. The Bausch & Lomb Research Lamp is specially designed to meet the requirements of the critical microscopist. Its source, enclosed in a light tight, yet well ventilated housing, consists of a 6 Volt 108 Watt Tungsten Ribbon Filament bulb which is used with a transformer on 110 Volt A.C. The bulb is of the prefocused type which eliminates tedious centering and alignment with the condensing system when lamps are changed. The lamp housing is fitted with a highly corrected condenser with iris diaphragm. The condenser can be focused by means of a lever. A support protruding from the front of the lamp house carries a water cooling cell and filter holder. Since it is necessary to control the intensity of the illumination to meet specific conditions a set of four neutral glass filters are supplied, having densities of 0.3, 0.6, 0.9 and 1.5 respectively. These can be used alone or in conjunction with Wratten or other filters. Best results are obtained when the source or Ribbon filament is focused sharply on the mirror of the microscope. The condenser of the microscope will then form a uniformly illuminated image of the front surface of the condensing system of the lamp in the plane of the object. When the specimen on the microscope is properly illuminated, the field of view will be completely and evenly filled with light if the object is viewed through the eyepiece. In addition, the light entering the microscope objective must completely fill the aperture of the objective. This may be checked by observing the back lens of the objective by removing the eyepiece and by viewing the back lens through a pin hole cap. It will be found that in order to secure the best results the condenser of the microscope should be carefully focused for each objective. In the case of an oil immersion lens, the back lens of the oil immersion should be completely filled with light, thus making certain that its numerical aperture is fully utilized. In the case of a dry objective (4 or 3 mm) the back lens need not be fully illuminated when working with lightly stained specimens as too much light tends to obliterate fine details in the specimen. It is suggested to reduce the iris diaphragm of the condenser of the microscope so that only 2/3 of the aperature of the back lens of such an objective is filled with light. When working with oil immersion lenses, care should be taken that the iris diaphragm in front of the condensing system of the research lamp is reduced as otherwise too much of the object is illuminated resulting in "glare". This field diaphragm should be wide open when low power objectives are employed. The full numerical aperture of an oil

immersion objective is utilized only when cedar wood oil is put between the front of the microscope condenser and the back of the slide. If this condition is not fulfilled, the numerical aperture of the observing objective is reduced to 1 as light leaving the condenser passes through air whose refractive index is 1 as against 1.5 of cedarwood oil.
(This contribution by Mr. E. H. Anthes of the Bausch & Lomb Optical Co. was prepared upon request of the editors)

Lebedeff, G.A. Method of mounting the reproductive organs of Drosophila. The fly is placed in a drop of physiological solution and the abdomen is separated from the rest of the body. With two needles the reproductive organs (duct and gonads) are cleared from the rest of the abdomenal organs. At this time it is advisable to transfer the organs to another drop of physiological solution (on the same slide) in order to eliminate small bits of foreign tissue which may adhere to the organs. Next, the organs are transferred into a drop of white of egg on to another clean slide (with shallow concavity) where the organs are placed in the desired position. Excess of albumen is removed by means of blotting paper. This transfer helps the organs to adhere to the slide and prevents their drying. After leaving the organs in the white of egg for about 12 to 24 hours, the slide is then placed into absolute alcohol, then transferred into solutions of 85, 50, 35, 15 percent alcohol for about 30 minutes or less in each. Then the slide is ready to be placed in tap water. If it is desired to have the colorless parts of the duct and the ovary differentiated, the slide may be stained with "light green". The testes, however, will preserve natural color. The preparation then is ready for dehydration and is passed through alcohol solution, ending with absolute xylol. Thirty minutes or less is a sufficient period of time for keeping the preparation in each of the alcohol solutions. Finally, the preparation is mounted in balsam or euparal.

EQUIPMENT

Thermal Control

Bridges, C. B. Incubators and thermal control. For incubators and cold-rooms of room size the least expensive and best engineered installation I have seen is that built at Stanford University, Palo Alto, under designs of Prof. Douglas Whittaker in cooperation with the Frigidaire Company. This controls against both rise and fall of temperature and its range of settings is from slightly above freezing to above 40° C.

Detailed descriptions of incubators and thermal controls used at the Pasadena laboratory and also at several other laboratories in the U.S.A. are given in: "Apparatus and methods for Drosophila culture". Am. Nat. 66: 250-273, 1932.

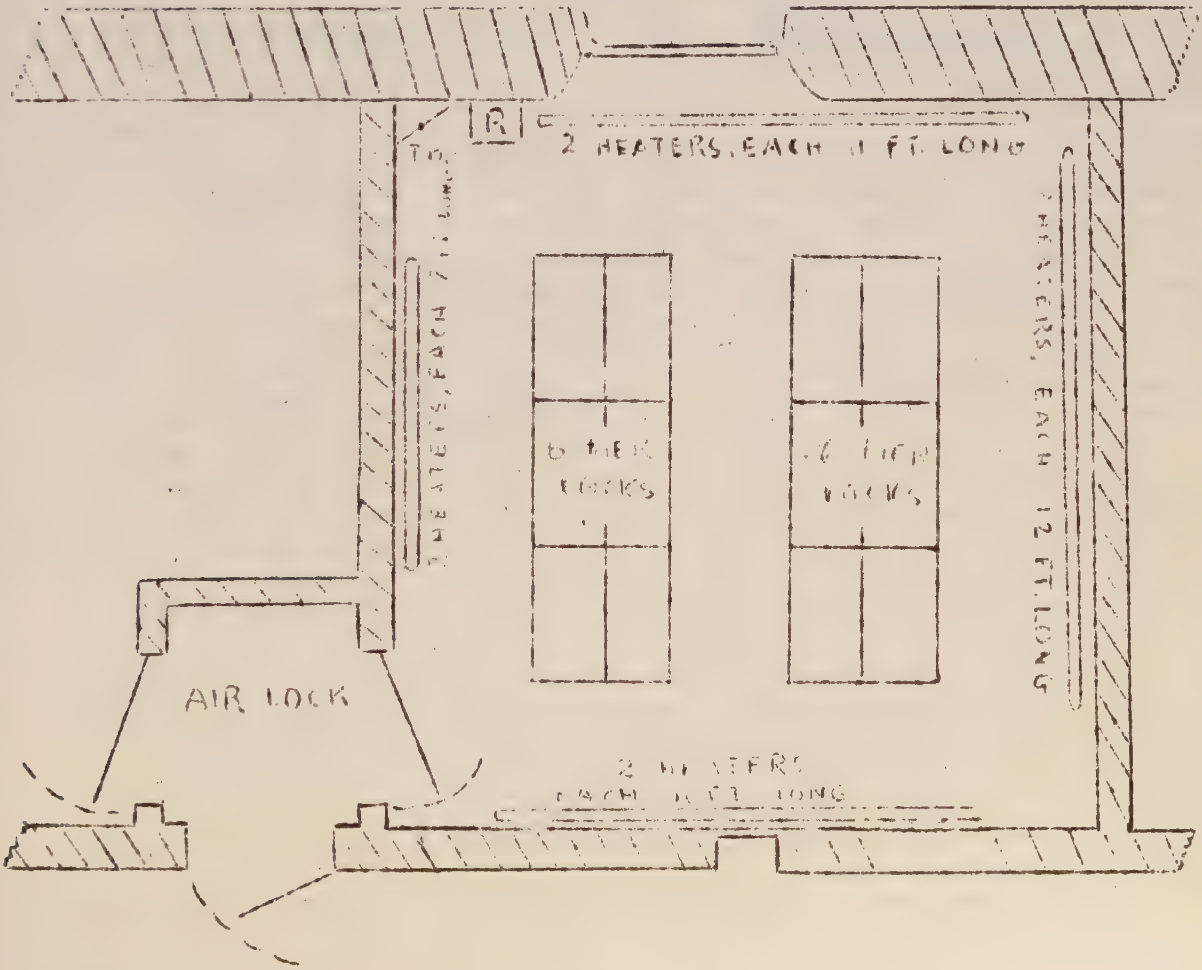
For trouble-proof inexpensive relay that described by Koepf and Mezen in Science 83: 109-110 would seem to be the best available.

Crew, F.A.E. Constant temperature room. The room measures 15' x 14' 9" x 8' 8" high. Three of the walls are inside ones and the outside one is to the South and has a thickness of 2'. The north wall is of 10" thickness having a corridor on the other side. The remaining walls are of 6" thickness. The rooms on either side and also the corridor have an average temperature of 14° C. The floor and ceiling are of aerro-concrete construction, and although the actual thickness was not ascertained for the purpose of arriving at probable heat losses, a value of 12" was taken. The window in the room is in the south wall and has a glass area of approximately 16 square feet.

It was estimated that 82 feet of 2" diameter tubular heaters loaded at 60 watts per foot run would be sufficient to maintain the temperature of the room at any predetermined value up to 28° C. with the outside temperature at 5° C.

The heaters are mounted horizontally in pairs on each of the walls at a height of 15" from floor level. At time of erection provision was made for adding an extra tube to each or any pair should it be desired to run at higher temperatures. As the supply available was 230 volts, 50 cycles single phase alternating current, it was possible to adopt a thermionic relay switch for the automatic temperature control of the room. The relay switch comprises a thermionic valve supplied by a small transformer with filament anode and grid potentials operating the triple pole main contactor switch by means of the anode current. The thermostat is connected in the grid circuit and effects control by the application or non-application of a suitable potential to the grid. The thermostat is similar in construction to a glass mercury thermometer but in addition contains two contacts one of which is in permanent contact with the column of mercury. The other is connected to a small spiral of platinum wire. A small glass capsule containing a short piece of iron is located at the top of the thermostat tube to which is attached a length of platinum wire passing through and making contact with the platinum spiral already mentioned. The capsule can be moved up and down in the tube by means of a permanent magnet applied externally and hence the thermostat can be arranged to make contact and "open" the main contactor switch at any desired temperature. With this type of thermostat it is possible to obtain temperature regulation within extremely fine limits.

On test it was found that in the event of the filament of the thermionic valve failing the main contactor switch would remain "on" and the room temperature rise to excessively high level. A small relay was therefore included in the filament circuit and so long as current is flowing in the filament the armature of the relay is held away from a pair of contacts, which when bridged by the falling armature completes a battery circuit to an alarm bell fixed in the corridor. In the event of an interruption of the supply, this relay also operates the alarm bell. (Messrs. Round & Robertson, Edinburgh).
See sketch


R
Th.
2 HEATERS, EACH 11 FT. LONG
2 HEATERS, EACH 7 FT. LONG
6 TIER RACKS
6 TIER RACKS
2 HEATERS, EACH 12 FT. LONG
AIR LOCK
2 HEATERS
EACH 11 FT. LONG

Gravett, Howard L. Thermal control system. Temperature is controlled with great exactness in the Experimental Zoology Laboratory at the University of Illinois. Electric incubators are housed in especially constructed high- and low-temperature rooms. While experiments are being run it is possible to keep these rooms from varying more than one degree from 25° and 15° C. Under these conditions the incubators housed in them can be held constant to within 0.3° of the required temperature.

The incubators used are made by the Chicago Surgical and Electrical Company. The heat unit is a high resistance wire coil and contact is accomplished by the bending of a diaphragm. A twelve-inch General Electric dish fan with lengthened shaft so that it could be installed with the motor outside and the blades inside of the chamber has acted in a satisfactory manner in keeping the air stirred. The only attempt to control the humidity is to keep an open vessel of water in each incubator. A Thycos, ribbon type, be-record thermometer made by the Taylor Instrument Company of Rochester, New York, is used for a continuous record of the temperature.

The constant temperature rooms are cooled by air blown over brine coils and the high-temperature room is varied by steam coils. Both systems are under automatic control installed by the Johnson Service Company of Milwaukee.

Department of Biology, Amherst College. Constant temperature and humidity control. With the assistance of a grant from the Rockefeller Foundation, the laboratory has now completed the installation of a constant temperature room especially desigened for Drosophila work. The room is about 15' x 8' x 8' and is built into a storeroom on the basement floor. The walls and ceiling are 3" cork insulation attached on the inside of 4" studding, and two coats of water-proof cement plaster are applied over the cork. The outside is covered with fir sheathing, so that a 4" air space is enclosed. The room is air conditioned by an air duct on the end wall, and outlets on the side walls. The air conditioning apparatus was furnished by the Carrier Co. and is capable of maintaining the room at any temperature from 5° C to 50° ± 2° C with a relative humidity of from 20% to 100% ± 5%. At present the room is being maintained at 15° C with a relative humidity of 65%. Water and electric current are available within the room.

Within the room are placed 12 unit incubators, similar to the two shelf type developed by Bridges and Plunkett. Each contains an 8" fan run at low speed. Temperatures from 16° C to 40° C can be maintained over long periods within any of these units. The heating elements are electric bulbs and the thermoregulators are toluol or alcohol filled, controlling the heating elements by single relays amplified by a vacuum tube. The relay apparatus was furnished by Lovett Garceau, Diamond Hill, R.I., at $20.00 each. The temperature at any point in these incubators varies not more than 0.2° C over an indefinite period.

The humidity is controlled by the percentage humidity in the room itself.

In addition to the incubator chambers maintaining temperatures above that of the room, there has been installed a large four door Frigidaire. Within this have been introduced five distinct insulated compartments with double glass doors (about 14" x 10" x 15") each with a heating element enclosed in lamp cylinder of asbestos. The temperature is controlled by toluol filled thermo-regulators operating the heating element through Dunco Relays (Struthers Dunn, Inc., Phila. #CS-1022-$11.00). The Frigidaire cooling system maintains a temperature of 4°-8°C around the insulated chambers, and temperatures up to 15° C are maintained within the chambers with a variation of less than 0.2° C over long periods.

The system is somewhat unique in that the room as a whole is held at an intermediate temperature with a series of 17 compartments at accurately controlled temperature above and below that point. Such a system is much less expensive than that of keeping the room at the lowest point, and the various intervals are more easily maintained. It has now been in operation for three months and seems to be entirely satisfactory.

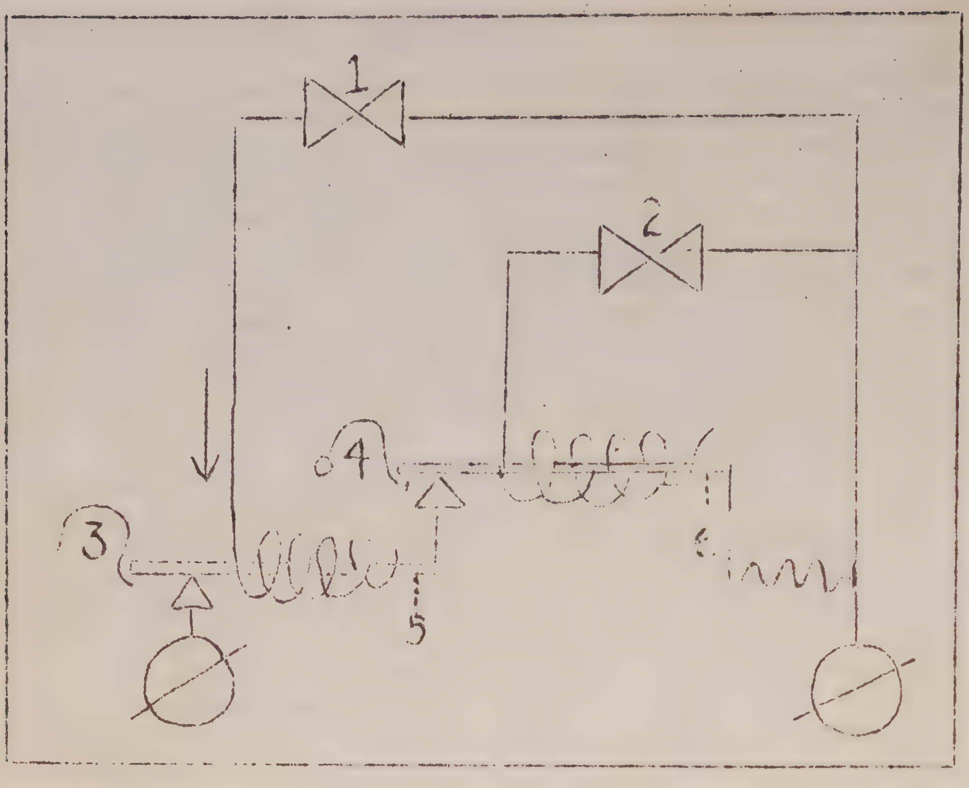
A series of Wheatstone Bridge Resistance Thermometers which will make a printed record of temperatures and relative humidities of six separate compartments simultaneously (with and accuracy of #0.25° C) is now being installed.

The room is large enough for a series of shelves and a table for microscopic work, so that observation as well as other types of work can be carried on.

The total cost of the whole installation was close to $4000.00. The room and equipment were planned by the members of the department. Further details can be furnished by writing to H. H. Plough.

Medvedev, N.N. Thermal control As experience shows, the best solution of the incubator problem is the construction of one big room-like thermostat instead of a number of them for personal use or for the use of some workers. The small incubators are used only in special work, when high or low temperatures are desirable.

The thermal control system of Frank that we adopted, after much experience, may be generally recommended as a very precise and cheap one. It consists of two regulators (see figure), one of which (2) keeps the desirable temperature (e.g. 26° C); the second regulator (1) is an extra one and breaks all contact when the temperature in the incubator accidently reaches 2-3 degrees above the point desired. This complete breakage of the current is brought about by the spring (3), being so designed that it breaks the current at a definite temperature but does not close it again automatically. The spring (4) on the other hand, breaks and closes contact automaticlly. Both regulators (1,2) as well as the breaking parts (5,6) are made from bimetallic plates.



Use of similar thermostat rooms and thermal control systems is reported by R. A. Masing and A. I. Zuitin.

Mossige, Jeanne Constant temperature arrangement. Constant temperature water baths up to 29° are used at the Zoology Laboratory of Columbia University with great success. The flies are placed in six inch vials in wire holders, the upper two inches above the water level, with deep cotton plugs. This gives a temperature accurate to within 0.1° and a larger working space than any of the usual small laboratory incubators.

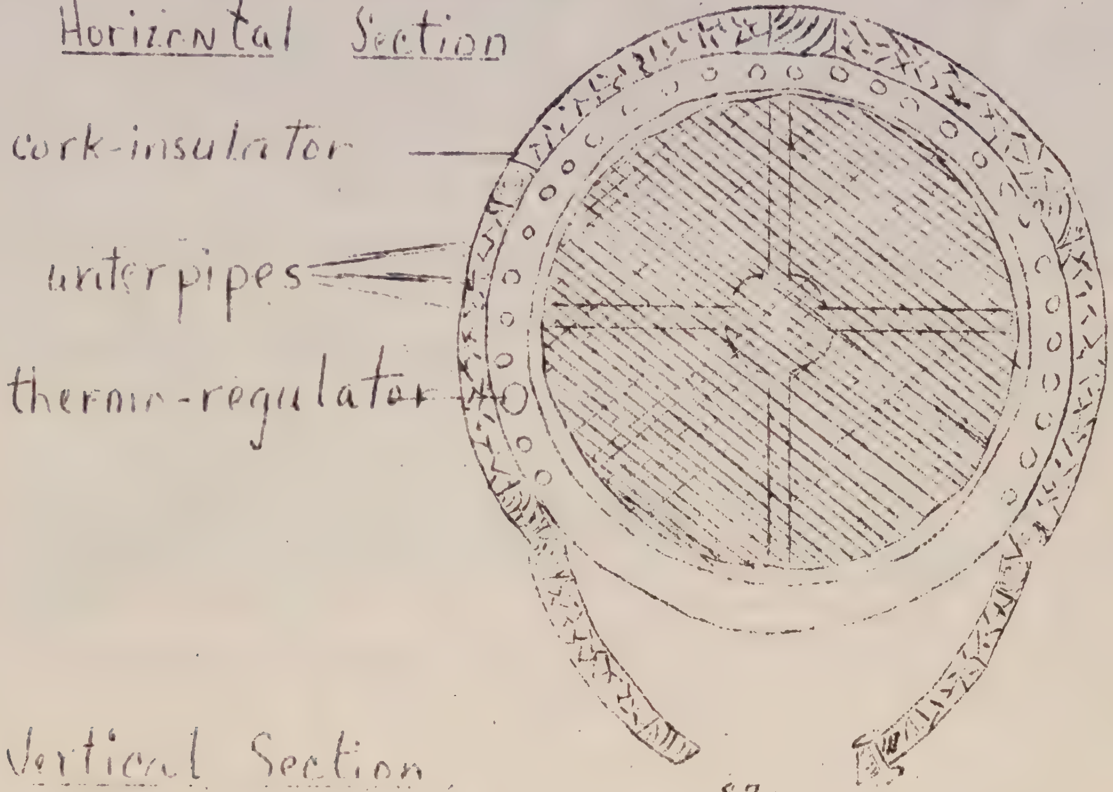
Shull, A.F. Heating units and thermal control. Heating units for temperature cabinets made up in any form, similar to those used in poultry brooders, may be obtained from Oakes Manufacturing Co. Inc., Tipton, Indiana. A small-sized unit costs $1.20.

Temperature control for these cabinets may be obtained with Circuit Breakers, made by the above company, at a cost of $1.30 each.

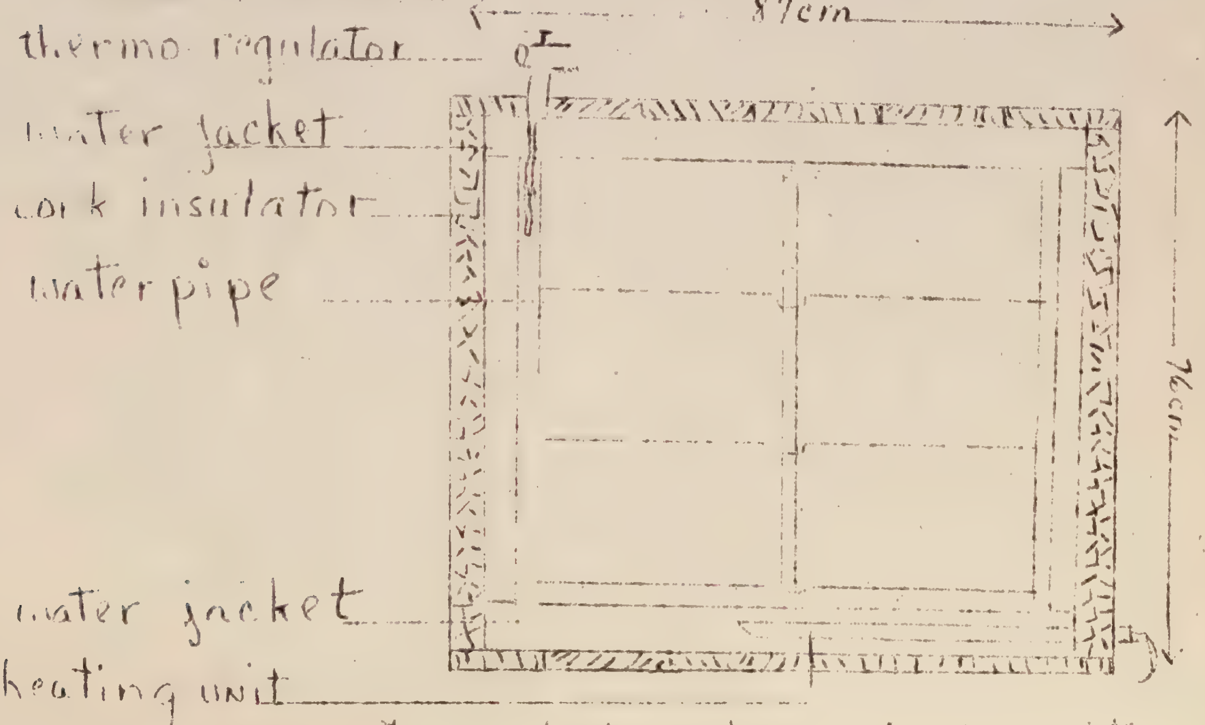
Camara, A. Incubators. We use Common incubators of Charles Hearson of London and Hugersboff of Leipzig. We have also some incubators built in this Institute.

Gottschewski, G. **Incubator.** The following figure shows the design of the incubator used at our laboratory. This incubator is built at our Institute. The capacity is 150 culture bottles. It costs about 600 R.M. including thermo-regulator which is obtained from Leitz, Cat. 32A no. 10190, Neuer Elektro-Präzisions-Thermoregulator grosses Modell and costs 98 R.M.

Horizontal Section



Vertical Section



(for temperatures higher than 30°C. two units used)

Hersh, A. H. Incubators. A multiple temperature incubator, total length about 15 feet including an icebox at one end and at the other an electrically heated water tank fitted with thermoregulator. A long copper trough insulated with glass wool serves for heat conduction. This apparatus is slightly modified from model #3 described by C. B. Williams and T. W. Kirkpatrick (1924, Technical and Scientific bulletin # 38, Ministry of Agriculture, The Government Press, Cairo, Egypt). The thick copper trough is deep enough to accommodate 8 oz. bottles. When the hot tank is kept at about 40° C, a temperature gradient over the interval 10° to 35° can be maintained with sufficient constancy for any investigation which does not require any extensive amount of space at any single temperature.

Spencer, W. P. Incubators In culturing many species of Drosophila optimum temperature conditions are extremely important and even in the case of easily cultured species such as melanogaster, virilis, or funebris much of the trouble with mold, mites, and infertility would be obviated by starting cultures at the optimum temperature.

The incubators described below are not presumed to take the place of the accurate and elaborate temperature control systems developed by Bridges and Plunkett. They do, however, supply at very little expense a means of rearing large numbers of stock and experimental cultures at a temperature fluctuating not more than a degree centigrade.

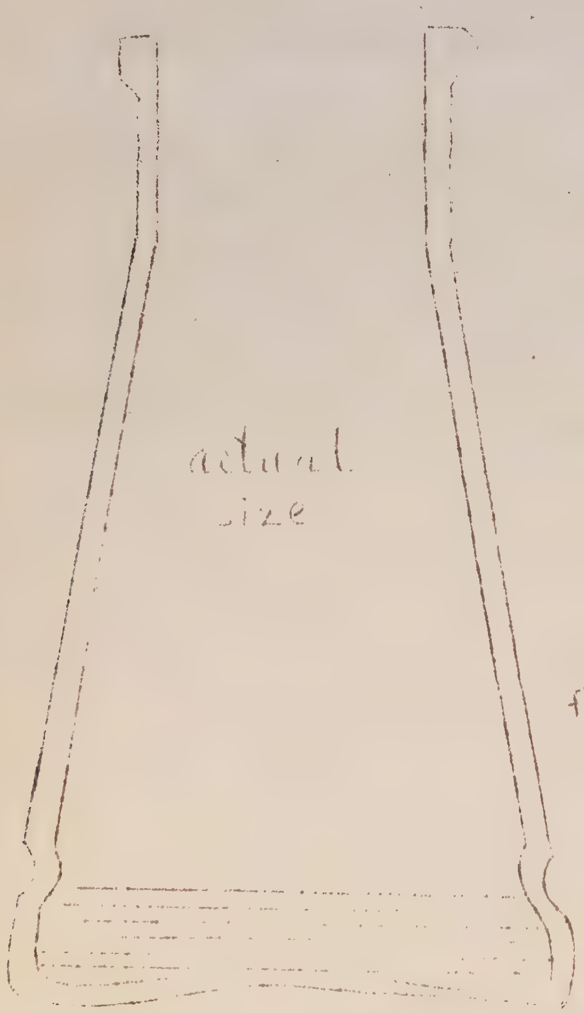
A casket pack (rough box) may be secured from any funeral director for from 25 to 50 cents. This consists of a strong light wooden framework to which is tacked three ply 1/8 inch wood veneer panels. These boxes vary in size; one of average size we are using has the following inside measurements: length 7' 4", width 2' 6", depth 2' 1". The lid is hinged to the box with three or four hinges, and hooks or other fasteners provided to hold this door shut. As the wood veneer is tacked on the inside of the framework, panels of cellotex or other composition insulating material are cut of a size to fit snugly into the framework on the outside. These are held in place by one or two light lath tacked over them and to the framework. The box is set on end and may be put on castors. The first shelf is placed at least two feet above the bottom. These shelves may be made of one-half inch wire mesh tacked on wooden frames which rest on supports nailed to the framework. Each shelf, for an incubator of the above dimensions, has a capacity of 100 half pint milk bottles. Five shelves can easily be used with ample room for removing culture bottles from the back of the shelf without disturbing those in front. The heating unit consists of electric light bulbs placed in or near the bottom of the incubator. A thermostat is placed on the back wall about middle of the incubator. One light bulb of 60 watts is capable of keeping this incubator with approximately 40 cubic feet of air space about three degrees C above the room temperature. We use a 60-watt bulb in series with a bi-metallic thermostat and other bulbs which may be turned on, but which are not in series with the thermostat. By carrying a light load on the thermostat danger of sparking and sticking is lessened; then if this does occur the temperature will not go so high that serious damage is done. It is of course desirable to use a room for fly culture where day and night temperature fluctuate to a minimum. A cellotex shelf is provided which may be fitted in at any level to cut down the size of the incubator when the full capacity is not needed. Such a 500 bottle incubator gives surprisingly little fluctuation in temperature from shelf to shelf corners. This may be due to the long distance from the heating units to the first shelf. A galvanized pan three inches deep covers the bottom of the incubator and is kept full of water. The incubator, when empty can easily be moved by one person. The total cost is less than $10.00. Such an incubator has been in use in our laboratory for two years, with no appreciable wear.

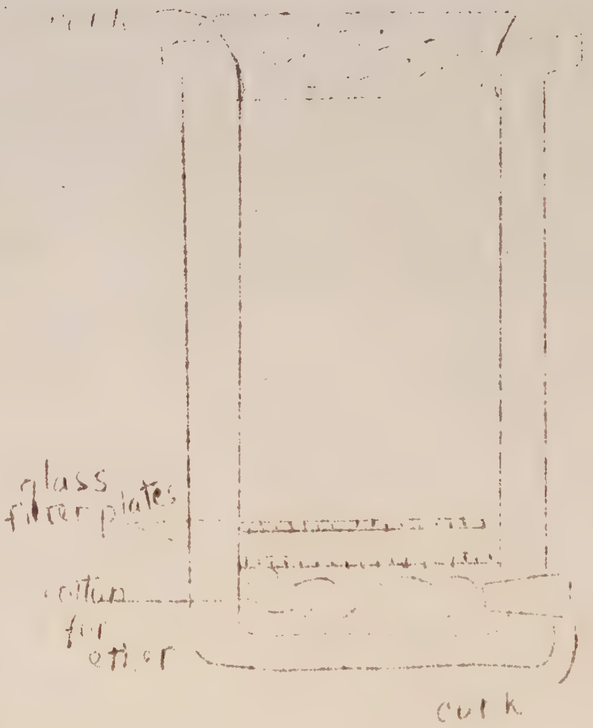
Stern, Curt Incubators. Two-shelf incubators with toluene mercury thermoregulator and Aminco relays as described by Bridges, 1932, Am.Nat. pg. 258-265. In order to keep temperature constant at or below outside temperatures (no cool rooms available !) we have installed cooling devices above the heating units at the height of the middle shelf: (a) copper tubing, through which running water circulates constantly is sufficient for keeping the temperature within a few degrees below the outside temperature, (b) for lower temperatures a grid connected with a refrigerator unit is being used.

Utensils

Crow, F.A.E. Culture bottles. Half-pint milk bottles are used for stock cultures. For convenience all experimental work is done in 4" x 1" vials.

Gottschewski, G. Culture bottle and etherizer. Culture bottles can be obtained from Bertsch, Quilitz u. Co., Berlin N.W. 40, Döberitzer Str. 3-4. Cost is 0.20 R.M. per piece. The etherizer is made by the Jenaer Glaswerk Schott u. Gen., Jena, and costs 8.50 R.M.





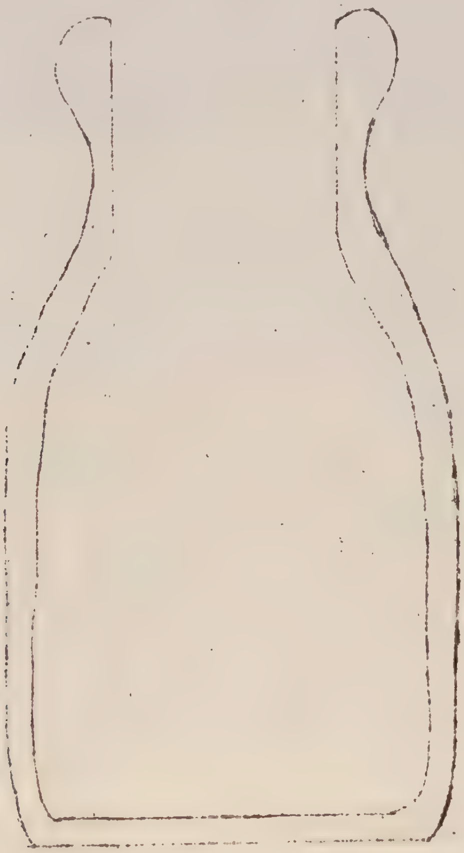
Kyoto University. Culture bottles Generally the bottles for pickling the scallion (allium bakeri) are used. The bottles are used widely in Japan, and can be purchased at a low price (about ¥4 per 100 bottles). The size and the volume of the bottle are as follows: Height, about 18 cm; inside diameter of the mouth, about 3 cm; inside diameter of the bottom, about 6 cm; volume, about 350 cc.

For special works, the small milk bottles or other adequate bottles and tubes are used.

Gravett, Howard L. Culture bottles. The bottles used for the various stocks are wide-mouthed 250 c.c. bottles. The dimensions are as follows: height (including neck) 6 inches, width at base and below neck 2.5 inches, neck 1 inch and width of neck 1.25 inches.

Hemopathic vials (3/4 x 3.5 inches) are used for experimental work in which single pair matings are to be used.

Medvedev, N.N. Culture bottles For individual crosses and tests we use vials 80 x 20 mm in size. In order to get a large number of flies we use a special kind of bottle, adopted in our Institute over two years ago. They are made of thick glass, preventing the accidental breadage of them. The size and dimension of this kind of bottle is given in the following figure.

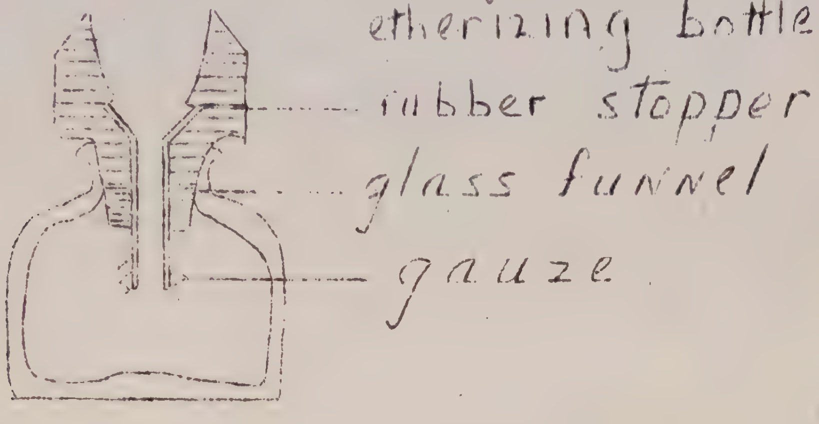


Shipman, E.E. Culture bottle. Due to the high cost of transportation on the bottles designed by Bridges and manufactured by the Owens-Illinois Pacific Coast Company at San Francisco, it was necessary to find a substitute bottle manufactured nearer home. The writer has found a Urine Specimen Bottle, No. 820, manufactured by the Glasco Products Company, Chicago, Illinois, quite satisfactory. The bottle is made of the same type of glass as milk bottles, has straight sloping sides, the inside top diameter is about 1/4 inch less than the inside bottom diameter, and has milk bottle type opening so that paper caps may be used if desired. The writer handled three gross of them this summer with an average of about 35 offspring per bottle and had only four cases where the food cake shook completely loose, daily removals were made so that the danger of loose food cakes was much greater than in routine stock work. (Copied from DIS-3: 54).

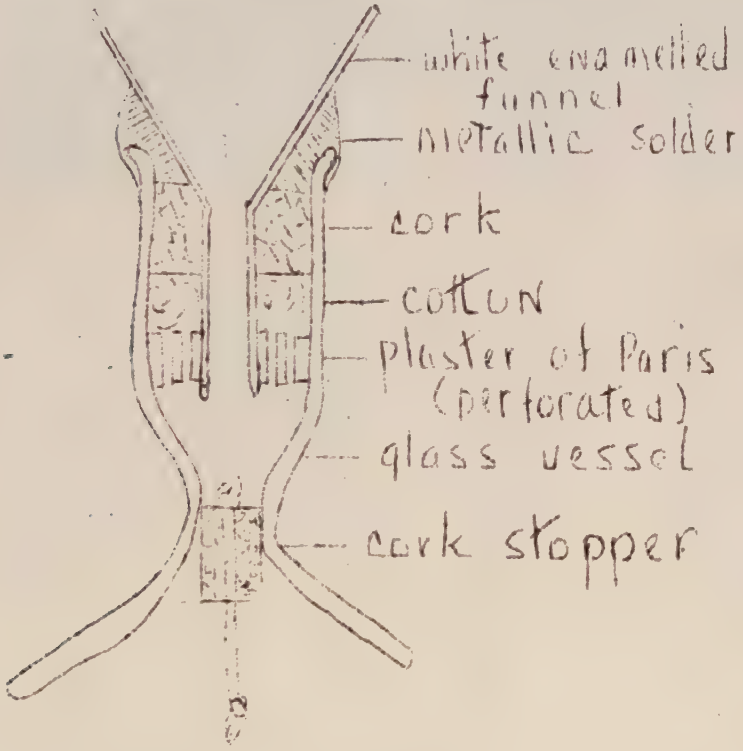
Spencer, W. P. Culture bottles. For some months I have been using small green and white glass salt and pepper shakers, with aluminum screw caps in place of glass vials for culturing flies. These shakers can be purchased for two for five cents in any 5 and 10 store in the U. S. They have a total capacity of 60 cc.; we use 15 cc. food medium in them as compared to 30 cc food medium in a quarter pint milk bottle and 50 cc. in a half pint bottle. As the holes in the screw caps are large enough to let small Drosophila through, circular disks are cut from library cards and fitted inside the caps, and then punched with needle holes. When once a cap is fitted with a card-board disk it can be sterilized and used many times before a new card-board disk is needed. An ordinary library card is cut in four pieces, 2-1/2" by 1-1/2", each piece is folded once and these are stuck, one into each culture bottle. The card serves a double purpose; it is sufficiently rigid to hold the food plug in place in case of CO_2 formation, as it pushes against the screw cap; then it furnishes pupation surface. A larger square bottle, with aluminum screw cap can be purchased for five cents each. This bottle occupies the same shelf space as a half pint milk bottle but has considerably more food surface. I am using the small shakers for rearing stock cultures of a number of the smaller Drosophila species as montium, bipectinata, and affinis, and for maturing flies of slow breeding species. Small tags made of strips of library card are used for numbering, and these tags are fitted under the edge of the screw cap. It is to be hoped that eventually square culture bottles, with aluminum screw caps punched with very fine holes may be placed on the market. Such bottles made of clear glass ought to sell for not more than five cents a piece for larger sizes and correspondingly less for small sizes. However, it is not likely that these prices can be secured without the cooperation of a number of the larger laboratories.

Amherst Laboratory Etherizing bottle. It has been found that a rubber Walter's Crucible Holder furnished by any laboratory supply house at thirty-five cents is an excellent stopper for the etherizing bottle (of sketch) and makes the use of more expensive and more complex bottles unnecessary. The rubber stopper fits around the lip of any bottle or vial, and flies shaken out fall through the glass funnel into the bottle. A few drops of ether from a dropping bottle on the gauze around the stem of the funnel is sufficient for several samples of flies. The outer lip of the rubber stopper should be ground down to form a narrow upper lip all around. The stem of the glass funnel is cut to a suitable length and fire polished.

Walter's Crucible Holder as stopper for etherizing bottle



Demerec, M. Etherizing bottle.



The following drawing represents an actual size cross section of the etherizing bottle used in our laboratory. The design is a slight modification of an early Bridge's design. Ether is poured in through the bottom hole. A few drops suffice for one hour's work.

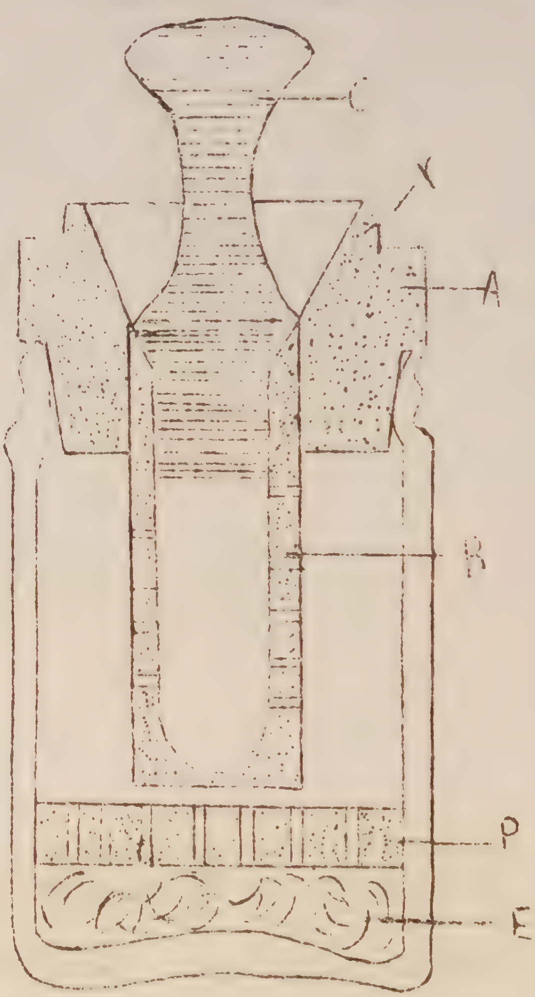
Specifications:
Funnel: White enameled 1/8 quart improved funnel manufactured by the Vollrath Co., Sheboygan, Wis., obtainable in hardware stores or could be ordered through a hardware store. Top is cut off to fit the culture bottle and bottom cut off to fit the glass vessel. Cost 30-40 cents.
Glass vessel: Can be made by any glass-blower. We ordered it from Eck & Krebs, 131 West 24th Street, New York, at 50 cents a piece. (Copied from DIS-2: 62)

Mickey, George H. Etherizer. A very simple type of etherizer may be constructed from a pure aluminum, seamless funnel, 2 in. across top, (may be purchased for about 75 cents per dozen at any hardware store) and a A.C. glass carbureter bowl (genuine A.C.

service part no. 854004, 15 cents each; A.C. Spark plug Company Flint, Michigan).

The top of the funnel must be trimmed down to a slightly larger size than the diameter of the cup then bent over the latter's edge. Around the funnel tube a mass of cotton covered with cheese cloth is wound tightly and held in place with fine wire or with string. Finally, the lower tip of the tube is slit in several places upward to the cotton and the resulting "fingers" bent up to help hold the cotton securely. This etherizer is probably best adapted to use in a laboratory where little fly work is done or to elementary students use, because of its simplicity and cheapness, but it is not suited to prolonged Drosophila work because of loss of ether.

Doubtless the improvement described by Stern in DIS-1 and by Demerec in DIS-2 on Bridge's original etherizer ('32, Amer.Nat. 66: 250-273), leave little to be desired in the way of an etherizer; nevertheless, through numerous experiments we have developed a new model based upon Muller's suggestions in DIS-2. The accompanying sketch represents an actual size section of our special design. The glass bottle is an ordinary specimen jar (Cenco no. 10373 wide mouth bottle, diam. 2-1/8", ht. 3-1/4"; screw cap size no.53; 40 cents a dozen) hence may be replaced very readily if broken. The upper portion, funnel and tube, is made of Bakelite turned on a small lathe. Part A is fashioned from Bakelite BT-45-005 at 60 cents a pound, and parts B and C are of Bakelite BT-61-893 at $2.75 a pound, (procured from the Bakelite Corporation, 247 Park Avenue, New York City). The latter grade is used for the tube and stopper because it can be polished to a highly transparent quality.



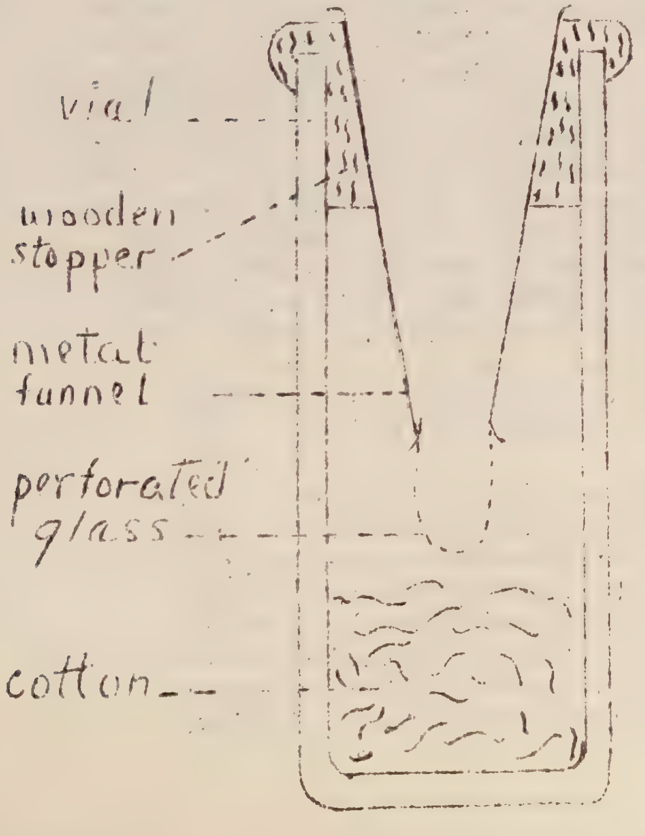
This screw cap jar has a diameter about equal to that of the mouth of a milk bottle and the threads give a nice grip, thereby making it convenient, after the milk bottle has been inverted over the rim (r), to hold it in place with one hand and knock the whole against a large rubber stopper.

Merely the friction of a tight joint is sufficient to hold the tube (B) in the top (A);

The bottom of the hole drilled in the tube should not be pointed but must be rounded so as to prevent flies being caught. Very small holes are drilled into the side of the tube (B) to allow ether vapor to enter from the glass jar. Cotton should be packed in the bottom of the glass jar (E) and held in place by plaster of paris (P) which should be perforated. If the tube is polished, it is possible to see the flies from the side and to tell when they are anaesthetized. Ether may be added either by pouring it into the tube or by dropping it onto the plaster after removing the top (A).

It is necessary to add ether only about once a day, or even at longer intervals if the stopper remains in place when not in use. Not only is the saving in ether remarkably economical but also valuable in regard to the comfort and well-being of the investigator. Indeed, this latter consideration was the chief reason for developing our new design.

Muller, H. J. Etherizing bottle. The type which I have found most practical all round (and at the same time the easiest to construct, repair and clean) consists of a glass containing-vessel (whose shape may be chosen according to the convenience of the operator), into the neck of which is firmly fitted, preferably through the mediation of a hollowed-out cork, a funnel, preferably of metal, with an upper end wide enough to fit against the mouth of the widest-mouthed culture vessel used. To the narrow end of the funnel, below, a large gelatine capsule of the same diameter, and containing numerous fine needle-holes, is glued on. In the space between the bottom of the suspended capsule and the bottom of the containing vessel is packed a mass of dense cotton, (which must not touch the capsule). Flies go in and out through the same opening, without manipulation of any stoppers being necessary and are quickly etherized by the diffusing ether. The ether used need usually be added to the bottle but once a day, as a minimum is lost (an advantage both economically and physiologically). If desired, glass can of course be substituted for the gelatine, but the latter has considerable durability and the ether can be poured in directly through the gelatine sieve. The latter is later wiped quite dry with a paint brush. (Copied from DIS-2:62



Spencer, W. P. Etherizing bottle. The bottle I am now using is an adaptation of the one described by H. J. Muller in DIS-2.

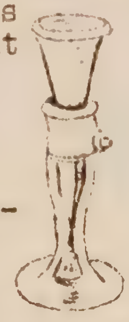
Wide-mouthed heavy glass, speciment bottle of about 60 cc. capacity; cork to fit. A hole 3/4 inch in diameter is cut through the cork with a large borer and then reamed out so that a large gelatine capsule, diameter 3/4 inch and length 1-7/8 inch can be fitted into the lower end of this hole. It is pushed in but not glued. With a hot needle numerous holes are punched in lower end of capsule. An aluminum funnel 2-1/2 inches long rests in the cork and sticks well down into the capsule. Cotton is packed in the bottom of the bottle and before using a pipette full of ether is introduced. Then the cork with capsule is inserted, the funnel laid in place and the flies jarred into the capsule. When they are etherized the funnel is removed and the flies poured out on the counting card. Etherization time is slightly longer than with the Bridges type etherizer. Simplicity of construction, ease of cleaning, and the fact that capsules are easily replaced are advantages. If the bottle is kept covered when not in use one pipette of ether lasts for hours.

Recently a workman in our laboratory constructed 15 of these etherizers in two hours time.

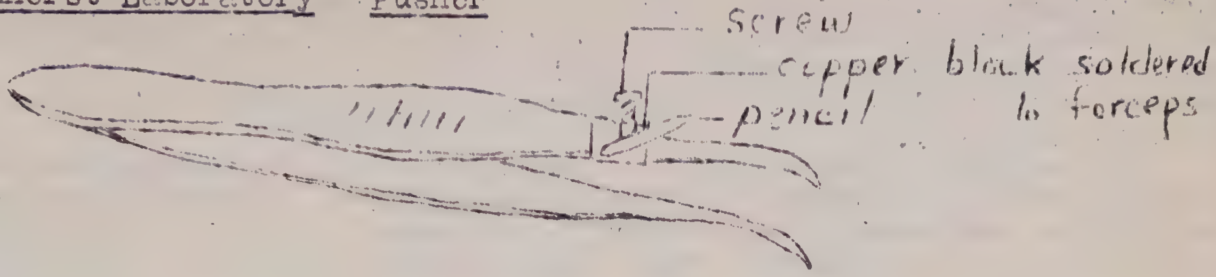
Total cost:
Construction time -- 10 minutes
1 large cork -------.02 cents
1 gelatine capsule -.02 "
1 alumin. funnel ---.10 "
1 specimen bottle --.15 "

Stern, Curt Food-filling funnel and etherizer. A funnel has been constructed which facilitates the filling of food-bottles. The funnel is made of metal and has a capacity sufficient for food for about 60 bottles. It has a metal faucet which is easy to handle. The funnel is mounted on a stand but can be detached for cleaning purposes. Below the funnel opening the stand has a hole through which extra drops of the food fall through, so that the bottles do not get dirty. The base of the stand has a rail which guides the bottles. This funnel can be obtained from the Will Corporation, Rochester, N.Y. A similar model has been in use for many years at the Kaiser Wilhelm Institut and has proven a great help in reducing the time and labor involved in the preparation of fly-food.

The Will Corporation has also on sale an etherizing glass following in general the design of C. B. Bridges (32) given in Amer. Nat., 66:250-273. An improvement consists of the following: Instead of a small funnel and a layer of plaster of Paris which have to be fitted and fastened into each glass individually, a one piece metal funnel and ether holder is used which fits in without special fastening devices. (Copied from DIS-1:57).



Amherst Laboratory Pusher



Forceps with pencil attached to speed up orienting fly and recording.

Mickey, George H. Pusher An excellent fly pusher may be constructed out of a metal needle holder (80 mm long, with screw chuck for holding needles; 20 cents each, no. 2978 Schaar and Company, Chicago), a steel spear point needle (40 cents doz., no. 3951 Central Scientific Company, Chicago), a piece of rubber tubing 1/8 in. inside diameter, and a no.3 or no. 4 red sable point brush (obtainable at any paoint store for 20-25 cents each). Red sable is little more expensive than camel's hair but is much more satisfactory in that it wears longer and has more "life" or spring. In place of the wooden handle of the brush one should substitute the metal needle holder, the back-end of which is held in place by any good metal cement. Then the piece of rubber tubing should be slipped over the handle to make a larger, firmer grip. The angle of the needle's spear point can be filed down to suit individual preference. When the needle is inserted, the pusher is ready for use.

Shipman, Emmet E. Pushers. In handling individual flies the writer has found a pair of regular dental forceps highly satisfactory. They are about 15 cm. long, the points being set at an angle are about 17 mm. long. The spring tension may be reduced by grinding on the inner part of the springs near the base. The writer has found them more comfortable for long use and the points stay in good alignment. They may be obtained from nearly any store handling dental and medical supplies.

Mickey, George H. Counting plate. Until recently at our Austin laboratories the most satisfactory plates on which to count flies has been old glass photographic or lantern slide plates which were out of date. At first they are rather too light in color, causing reflection and glare, but gradually become darker the longer they are exposed. Disadvantages are that the color finally turns too dark, the emulsion wears off through sliding on the table top, and the plates are broken too easily. We have just received some baked enamel plates from the Burdick Enamel Sign Company, 36 South State

Street, Chicago, which have none of these disadvantages. These new plates, made of 18 Ga. steel 3 x 5 inches in size, either have both sides covered with a medium dark gray baked enamel or have one side white and the other gray. In lots of 24 the price is 75 cents each.

(A plate of opaque glass was found to be a very satisfactory counting plate. It gives a rigid, smooth surface of good optical quality for examining flies. Such a plate can be cut to any size. Opal glass can be procurred from various supply houses. - Editors).

Muller, H. J. Fly morgue In place of the usual method of having a jar of alcohol or other volatile fluid into which the flies to be discarded are dropped through a narrow slit, it is much more convenient to have a broad dish containing a non-volatile oil. The used oil from automobiles affords a conveniently obtained medium. The opening may be protected by a wide-mesh wire grating. The flies do not have to be brushed off in any exact manner, but may be merely jarred off by knocking the porcelain plate against the screen with one motion of one hand. Renewal is seldom necessary and there are no disturbing odors. This method was used independently in Texas and in the USSR.

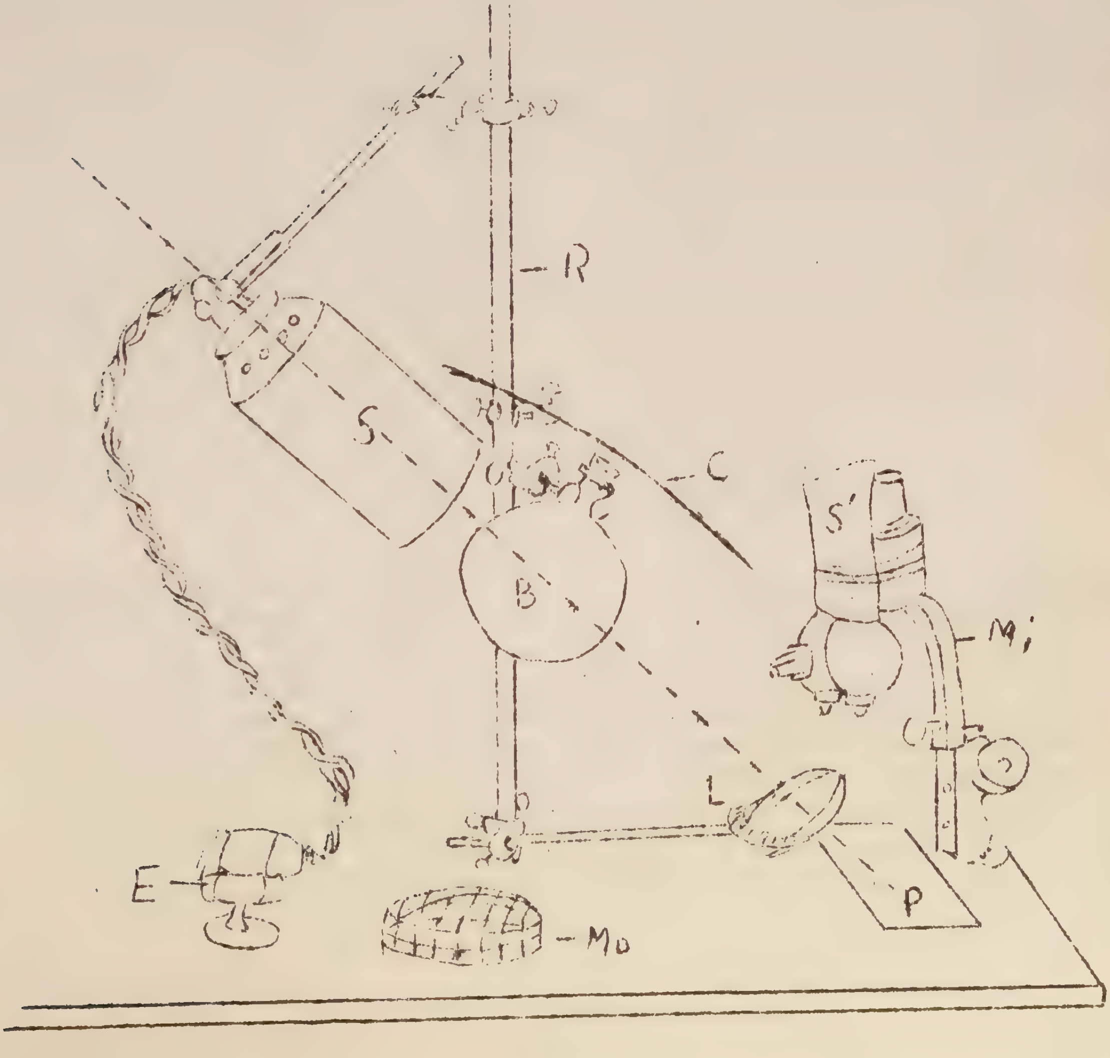
Mickey, George H. Trays for vials A very satisfactory container which has not been reported to DIS but which has been in use for handling flat bottomed shell vials, is a round tray 11 in. across and 3 in. deep, made of 20 Ga. (or lighter) galvanized sheet iron. Such a tray can be washed and sterilized repeatedly and will last indefinitely. Since it holds approximately 100 vials, no more convenient method for handling and filling vials without actually counting them could be desired. Moreover, this container lends itself admirably to a method suggested by Altenberg of etherizing cultures "en masse". For this, the vials are inverted in one tray, covered with a towel half-saturated in ether, and then covered with another tray. There is very little danger of over-etherization and the flies do not stick in the food since the vials are inverted. These "tins", as they are called, were constructed by a local tinner at a cost of 50 cents each.

Bridges, C. B. Light Light for the binocular should fall on the flies and white-glass plate as a broad-base converging beam whose axis is about 45° from the vertical, which angle gives least highlights and disturbing reflections. A frosted 100 W tungsten globe focused through a 1-liter globular water flask, placed very close to the flies, is excellent.

About 15 diameters seems to be optimum magnification for routine binocular examination of Drosophila, since higher magnifications have so little depth of focus that special or continual refocussing is necessary.

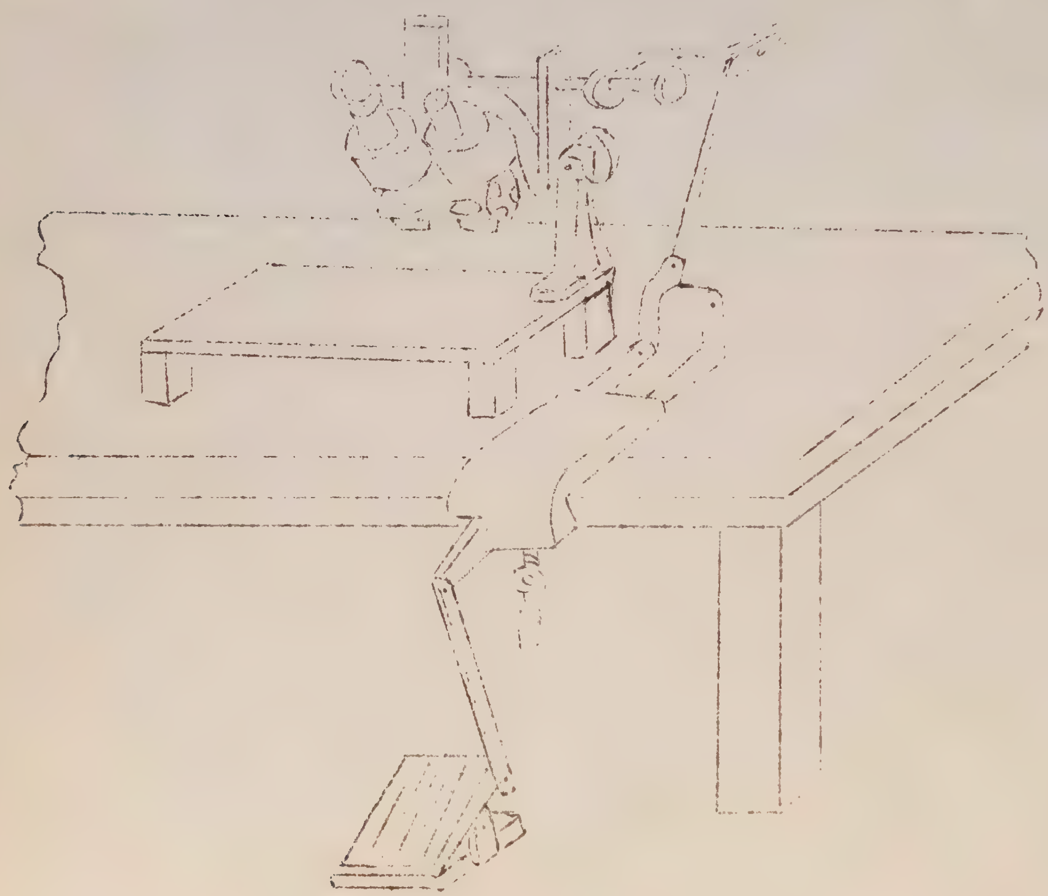
Mickey, George H. Lighting arrangement. Our present arrangement for dissecting binocular, lights, etc. is as illustrated in the following sketch. Mi, microscope; P, enameled counting plate; Mo, morgue, composed of finger bowl containing used crank case oil and covered with wire test tube holder cut down; E, electric socket on table top; R, ring stand screwed into hole in table; B, 500 cc. round bottom pyrex flask with short neck, filled with glycerine (much better than water) for cooling and concentrating the light rays; S, shade of bright tin (enameled black on outside) inside of which is a 200 watt frosted light bulb; C, cardboard to throw light down; S', small black shade on microscope to shield eyes from light.

Parts S, B, and L should be perfectly aligned and adjusted to make a field of light about 2 in. in diameter immediately below binocular.



Stern, Curt Foot-focusing device for binocular.

A special foot-focusing device for binoculars has been constructed by Bausch & Lomb, Rochester, N.Y. A simple system of levers connects a foot treadle with the focusing adjustment screw. It works with very little friction. This device proves highly useful whenever it is desired to use both hands for handling of the flies, e.g., in work with somatic segregation or in operative work with any object. The device can be adapted to different binoculars. The binocular used in Rochester is constructed, with certain changes, according to principles suggested by C. B. Bridges.



Oliver, C. P. A dry heat sterilizer for cotton plugs.

Where sterilizing equipment is not handy or not available, a small dry-heat sterilizer can be made at little cost. Heat is supplied by a common electric hot-plate (600 watts). To keep the cotton from scorching and yet to keep most of the heat from being lost, put a ten-inch-deep bucket between the plate and the sterilizing chanber. The open end of the bucket should be placed on the plate and should have a diameter as large as that of the electric coil. A large hold should be cut into the bucket-bottom, and it is better to have most of the hole covered with a strip of thin asbestos. Eight to ten small holes cut into the side of the bucket give the necessary circulation. A common, tin bread-box (10 x 12 inches) with the tip filled with numerous holes made with an ice pick serves as the sterilizing chamber. Cotton placed on the bottom of the box will scorch if the temperature goes too high. To eliminate this condition, cover the bottom of the chamber with a piece of thick (8 mm.) asbestos, supported at two ends by pieces of asbestos. Asbestos also lines the lower parts of the sides of the box, although this may not be necessary. Into a hole in the top of the box thrust a thermometer down into the mass of cotton. Heat easily reaches 65° C, and the cotton does not burn or scorch.

BREEDING METHODS

Culture media

Bridges, Calvin B. Food formula The food formula in use in the Pasadena laboratory by all workers is that published in Amer.Nat. 66: 268, 1932.

Water......................	75.0 c.c.
Molasses (free from SO_2	13.5 c.c.
Cornmeal (coarse yellow)	10.0 grams
Agar-Agar	1.5 grams

About 60 c.c. per 1/2 pint culture bottle.

Other recommendations in the above paper in Amer. Nat. still hold substantially correct.

Gottschewski, G. Culture medium. Normalfuttermenge für ca. 60 Kulturflaschen.
2000 ccm H_2O und 30 g Agar kochen lassen, bis Agar gelöst, dazu 125 ccm H_2O und 375 ccm Sirup. Schliesslich 600 ccm H_2O und 400 g Maismehl zusetzen, das Ganze zwei bis drei mal aufkochen lassen, beim letzten Mal werden 1-3/4 g Nipagin-M hinzugefügt (Nährmittelfabrik J. Penner A.G. Berlin-Schöneberg; konservierende Wirkung verhindert das Schimmeln der Kulturen). Das Futter wird dann inFlaschen (vgl. Fig. 1) abgefullt; dazu kommen einige Tropfen milchiger Emulsion von Bäckerhefe in H_2O gelöst, Krepp-Papier und Zillstoff-Wattekorken. Vor dem Abfüllen wird alles in 150° sterilizíert (elektrischer Sterilisator). Koch- und Abfüllgefässe (über letztere vgl. DIS-1:57, Apparatus, C. Stern) sind so bemessen, dass gleichzeitig eine Futtermenge für 300 Flaschen gekocht werden kann. Bis zum Gebrauch werden die Flaschen in Eisschränken aufbewahrt.

Gravett, Howard L. Food formulae The banana-agar-yeast food is used for most of the experimental work. The formula for the food is as follows: 100 c.c. of soft water, 100 grams of well-ripened banana and 2 grams of agar-agar. When the food is cool, a small pinch of pulverized yeast foam is added. It is then allowed to stand at 25° for about 24 hours before using.

The cornmeal-sorghum-agar food is used for maintaining the fly stocks. The formula used in the laboratory is as follows: 1155 c.c. of water, 22.5 gm. of agar-agar, 210 c.c. of sorghum and 120 gm. of cornmeal. One thousand c.c. of water is boiled and the agar added. The remainder of the water is mixed with the cornmeal to which the sorghum has already been added. This latter mixture is then added to the water and agar, stirred well, and placed in bottles.

Wilbur M. Luce adds 5 c.c. of sorghum to the regular banana-yeast-agar food and finds it very satisfactory for experimental work. He also tilts his vials after placing food in them in order to allow it to harden on a slant. He claims this lessens the possibility of gas formation and also increases the surface for yeast growth. (University of Illinois)

Kyoto Laboratory **Food formulae**

Agar-agar	10 gr.
Koji (malted rice)	100 gr.
Brown sugar	40 gr.
Acid potassium phosphate (KH_2PO_4)	0.8 gr.
Water	800 cc.

Use 50 cc. per culture. Heat the agar in the water (about 600 cc.) to dissolve, add the Koji ground in a mortar, with the sugar, the phosphate and the remaining water. This medium developed by M. Chino gives satisfactory results for D. melanogaster, D. virilis, D. ananassae and many other species. But, for D. repleta, D. buskii and a few other species, it seems proper to add albuminous material such as peptone in the above medium.

Ludwig, W. **Food Formulae** a) Standard-Nährboden für D. melanogaster:

Rüben-sirup 125 + Wasser 710 : Agar pulv. 10 erhitzen bis.z. Kochen; dann + Nipagin 1 g in 5 ccm Alkchol absol., dann Maismehl pulv. 133 + Wasser 200; weiter erhitzen bis z. Aufkochen. (Nipagin, erhältlich bei Julius Penner A.G.Berlin-Schoeneberg, verhindert in dieser Konzentration jede schimmelbildung).

b) Für D. funebris immer, für D. melanogaster gelegentlich, wenn Gläser mit Standard-Nährboden zufällig nicht vorrätig-sind: ein als Pulver Käuflicher Nährboden (bei Dr. P. Kuliga, Luisenkrankenhaus, Schumannstr. 78, Dusseldorf, Deutschland). In pulverflaschen 3 cm hoch auffüllen, mit wenig Hefosuspension, die 1% Nipagin enthält, kalt anrühren. Gläser durch Gaze, nicht curch Watte verschliessen. (Zusammensetzung des Nährbodens ist Geheimnis des Herstellers). Gläser sofort gebrauchsfertig.

Masing, R. and A.I. Zuitin
Food formulae The food formula used in our laboratories is as follows:

Agar-agar	40 gr.
Yeast	150 "
Currant	40 "
Sugar	50 "
Wheat flour	50 "
Water	750 "

Medvedev, N.N. **Food Formulae** Two kinds of fly food are used in our Institute. One of them is prepared for stocks exclusively; the formula of this kind of food is as follows: water 12.800; cornmeal 1.6000; syrup 1.400; agar-agar .200.

The second kind of food as reported by Offermann and Schmidt in DIS-3 gives the greatest number of flies per bottle. For the formulae see following article.

Offermann, C.A. and I.K. Schmidt
Culture media for Drosophila.

With the development of the Drosophila technique, not only a certain amount of sterilization of the culture medium during its preparation became necessary, but also an adaptation of it to different requirements. Productivity and duration of the media are the two main factors to be considered for our purpose, and they are to a certain degree in inverse relationship. By productivity we mean the quantity of flies produced in a given time. By means of overcrowding a certain food can yield a higher number of flies which are small in size, but this higher yield will usually be cancelled by a serious loss in the speed of development (in strongly overcrowded bottles in fact the cycle has proved to be as much as twice the usual length). Three functional types of media may be distinguished: 1) for the maintenance of parent flies, 2) for the maintenance of lines of stock cultures, 3) for the attainment of high productivity. 1) this type has proven to be extremely useful for the current work where we have to keep alive the flies from the moment we obtain them until the moment of their use. In this case offspring are not desired. Flies have been kept on such a medium for over a month (some over two months) at room temperature, without a transfer. The same vial or bottle can be used over again until the surface dries out, and etherized flies will not stick to its surface. - Water 90 cc., agar 2 gr., syrup 7 gr., Nipagin .15 gr. 2) Suitable media serving this purpose, such as the banana agar and the cornmeal syrup media, are already in use in all Drosophila laboratories and will not be described here. 3) The main characteristics of this type are: production of large quantity of flies, short cycle of development, and low selective level (preservation of individuals of low viability).

The addition of killed yeast in large quantities to the ordinary food formulae was introduced a few years ago by Muller (in 1928), giving surprisingly good results. These media had, however, the inconvenience or requiring a constant supply of fresh ingredients. Dry yeast was used in place of fresh yeast by Winchester and by Gershenson. The authors have recently experimented with a systematic series of modifications of the Russian food mixture with the addition of dry or fresh yeast. Fifty different modifications have been tried, approximately twenty vials being employed for each trial and counts of the offspring made. Each ingredient was tested in different concentrations. As a result the following formulae have been found the best for obtaining high productivity. (A. with dry yeast) - Water 80 cc., Agar 1.5 gr., Dry yeast 1.5 gr., Raisins 4 gr., Syrup 5 gr., cornmeal 5 gr., Nipagin .15 gr. The agar is dissolved by bringing the water slowly to the boiling point, dry yeast (that has been disintegrated in a small part of water) is added and the mass is kept boiling for another ten minutes, so as to make sure that all the yeast cells are killed. Then the mashed raisins, syrup and cornmeal are added. with continuous stirring, and the food will be ready for distribution. The addition to the liquid mass of "Nipagin T" Nachmittelfabrik Julius Penner A.G. Berlin-Schoeneberg as found in Dr. Nachtsheim's laboratory, is important for cultures which contain few larvae or develop slowly.

The layer of food should be somewhat deeper than 1/2 inch and its surface seeded with pure live yeast (fresh or dried). Adding paper and making the surface appetizing with fruit juice did not increase the yield in our case. 200 flies per vial and 1000 per half pint bottle should be considered a good average. This means that a vial can be employed where formerly a bottle was required, and a bottle can take the place of a group of bottles. Not only the number, but the size of flies is considerably increased. When fresh yeast is easily available it can be employed advantageously by substituting 15 grams fresh yeast for 1.5 grams of dry yeast in our formula.

The preceding formulae enable us to prepare food of each of the three types by the use of ingredients which will not spoil. A laboratory can thus provide itself with a year's supply at once, avoiding further trouble in this connection.

We desire to call special attention to the convenience offered by the new type of medium here described: The syrup-agar for the preservation of the P. flies, for the great elasticity it introduces in current laboratory work.

Parker, D.R. Food formula The food used in the University of Texas laboratory is made according to the following formula:

1 pound bananas (250 cc)
20 grams agar-agar
125 cc Karo syrup (white)
15 grams dried brewer's yeast (sterilized)
625 cc water
.15 gram Moldex A (dissolved in 95% alcohol)
Total 1 liter of food

The water and agar are heated until the agar is completely dissolved. When the bananas have been mashed thoroughly, they are added along with the other ingredients to the melted agar and the food is poured immediately, a glass funnel with rubber tube and spring stopcock being used for this purpose. Bottles are plugged with cotton stoppers, whereas the vials are covered with cloth towels until cool, after which they are sprayed with a thick suspension of fresh Fleischmann's yeast, punched, papered, and stoppered in the usual fashion. (According to suggestions by Muller in DIS-3: 52) Bottles are treated in the same manner; when they are cool, the stoppers are removed and paper toweling is added in the place of confetti. In both cases, by using a glass tube about 1/4" inside diameter and a large rubber bulb, a small hole is punched in the food at the side of the container in order to release gases formed in fermentation.

Spencer, W.P. Food formula

1 liter water
25 grams chopped agar
1000 grams mashed ripe or over-ripe banana.

It has been my experience that this medium is distinctly superior to corn-meal-molasses-agar for many species of Drosophila, and equal to it for all species tested, which will grow on corn-meal.

Tanaka, Yoshimaro Food formula

Wheat flour	70 gms
Brown (or raw) sugar	60 gms
Agar-agar	15 gms
Water	1000 cc

Synthetic Food (From Pearl, R. and U.B.D. Penniman, Am.Nat. 60: 357-366. 1926).

Solution A:

Cane sugar	500 gms
$KNaC\ O_6\ 4H_2O$	50 "
$(NH_4)_2\ SO_4$	12 "
$MgSO_4\ 7H_2O$	3 "
$CaCl_2$	1.5"

H_2O to make 3000 cc. of Solution

Solution B is composed of:

Agar-agar	135 gms.
Tartaric acid ($C_4H_6O_6$)	30 "
KH_2PO_4	4 "

H_2O to make 3000 cc. of Solution.

To prepare solution B, agar is thoroughly dissolved in water, to which the salts are added. To make the medium equal parts of solution A and B mixed. If a softer food is desirable the amount of agar should be decreased.

Gravett, H.L. Brewer's Yeast The following were the most favorable prices from a series of letters sent out last May.

1. Standard Brands Incorporated, Dry Yeast Department, 595 Madison Avenue, New York, New York.

Fleischmann's Pure Dey Brewer's Yeast - powered - supplied to universityies and colleges for 30 cents per pound, delivered, in quantities from 1 to 99 pounds.

2. Northwestern Yeast Company, 1750 North Ashland Avenue, Chicago, Illinois.

Yeast Foam Tablet Powder to universityies for 20 cents per pound in quantities up to 10 pounds.

3. Vitamin Food Company, Inc., 122 Hudson Street, New York, New York.

Vita-Food Dried Brewer's Yeast - No. 2 Green Label, a straight dried brewers yeast with hop taste, 1 pound cans at $3.90 per dozen (approximately 32-1/2 cents per pound). Prices F.O.B. New York City in quantities of less than 100 pounds. The prices are relatively lower on larger quantities. (University of Illinois)

Muller, H.J. Seeding with yeast. In place of the usual method of allowing drops of yeast to fall into the bottle from a pipette or sprinkling crumbs of yeast, it saves time and ensures more even distribution if one makes up a very thin suspension of the yeast in water, and then sprays this through a simple atomiser, such as is used for spraying fixative on charcoal drawings. In this way a great number of cultures may be seeded at once en masse.

Control of mites and molds

Spencer, W.P. Life history of the laboratory mite. The adults live in the culture medium and do not crawl over the inside of the culture bottle or on pupation paper as do the non-parasitic mites. The males are much smaller than the females, and may be seen mating with them; both have a squat appearance in contrast to the long, thinner non-parasitic mite. The eggs are laid in the culture medium and the young mites after several days to a week metamorphose into the migratory stage. These are brownish in color, just visible to the naked eye, and are extremely active. They crawl up out of the culture medium onto the sides of the bottle, and readily penetrate cotton plugs or other stoppers. They are constantly on the move and may travel at least several feet. During the course of this migration they attach to any insect with which they come in contact, and sink the mouth parts into the insect. On Drosophila they tend to attach themselves most frequently to the legs, particularly the proximal joints, to the wings, and about the genitalia. However, no part of the fly is immune and in heavy infestation literally hundreds of mites may attach to a single fly. Most of these mites leave the fly in a week or ten days after attachment, then grow to the adult stage in the culture medium and reproduce.

In fast breeding species of Drosophila such as melanogaster or simulans it is quite feasible to use mite infested parents and by taking their first offspring (providing temperature and other culture conditions have been optimum) get rid of the mites in one generation. This assumes that precautions have been taken to keep any new migrants from getting into the culture bottle. The life cycle of these species is more rapid than that of the mites and it seems that mites which have become firmly attached to one host do not leave for a second host.

In slower breeding species the best procedure is to transfer parents one to several times until they are finally free of the parasitic stage. One transfer is not always sufficient as a heavily infested fly may carry some of the parasitic stage for over two weeks before they leave, and in the meantime the mites which were first to leave will have produced a new generation of the migratory stage.

Spencer, W.P. Mite and mold control 1) The life cycle of mites seems to be less affected by low temperature than is that of flies. Hence during a mite infestation it is important to provide the optimum breeding temperature for each species of Drosophila and for all stock cultures suspected of being exposed to infestation. In general temperatures of 24 C to 26 C are optimum for most species, with certain exceptions noted in the literature. Funebris, hydei, immigrans do better at 24 C, melanogaster, simulans, carribea at 25 C or 26 C.

2) Rapid turnover of stocks with immediate discarding of all old stocks. During periods when mites are present all old

stocks are placed in a dry sterilizer and heated sufficiently to kill mites and eggs before bottles are washed. This seems a surer way of killing all mites than using a disinfecting solution on bottles.

3) Flies to be used as parents for new stocks are placed in small vials for one week to ten days, even though they appear to be free of mites. Then they are transferred to fresh stock culture bottles of standard size. During this period the mites which may have been present on the flies will have left them to breed in the culture medium, but a new generation of the migratory stage will not have come on.

4) All cultures, both the vials, and final stocks are kept standing in shallow metal trays containing Lysol solution, 1 part to 200 parts water. These trays are placed in incubators (see below) of 40 cubic feet capacity. As strong carbolic acid fumes are detrimental to flies it is is important to use as weak a Lysol or carbolic acid solution as will be effective. The above solution, 1:200 is not detrimental in enclosed incubators with evaporating surfaces of 1 square foot to two cubic feet of air space and no ventilating fan.

Tests of the following as possible liquid media for the control of mites were made; coal oil, 10% sulphuric acid, No. 10 light motor oil, 5% copper sulphate, Lysol. The migratory stage crawls readily through or on the surface of all of these except Lysol, which is very effective as a lethal agent. They also crawl readily through a band of vaseline. Many mites were then immersed in various concentrations of Lysol; the cessation of movement of all appendages was taken as the killing time.

Table

Dilution of Lysol	Killing time, All mites dead in
1: 40	5 minutes
1: 80	10 minutes
1: 160	15 minutes
1: 320	50 minutes

5) When a mite infestation seems to be entirely cleared up a fair sample of culture bottles from various incubators should be kept over a long period of time (isolated of course) as tests. The adults of the parasitic mite can readily be seen in such culture bottles and distinguished from the non-parasitic species of mites which may be present, but which can hardly be considered a pest. The parasitic mite in the adult stage is larger and with a squatty body, the non-parasitic mite is long and with long white hairs on the body.

6) Assume that any shipment of flies from another laboratory contains mites; of course this works both ways. It has been my experience that flies taken in nature seldom harbor the laboratory mite. In a considerable amount of collecting I have found them only once. Of course in mite-infested laboratories stray flies will likely be infested.

7) During a period of mite infestation all apparatus used in handling flies and all table tops etc. should be frequently wiped clean with Lysol or other sterilizing media, and it should

be kept in mind that mites can easily be passed from bottle to bottle by handling.

Most of the trouble with molds can be avoided by starting cultures at the optimum temperature until larvae are present. Of course in slow breeding species such as sulcata or repleta parent flies should be matured in small vials for a week to ten days. If a multiple mutant stock, or any one difficult to carry becomes infected with mold or harmful bacterial growths, flies to be used as parents from this stock may be kept for a few days in a vial with a hardy culture of some other species. Here the larvae of the second species keep down the mold and the flies of the first stock have a chance to mature in a mold-free environment. Assume that mold spores will be spread by infected etherizing bottle, or any other piece of apparatus used in manipulating flies and take necessary precautions to prevent this.

Demerec, M. Control of mites. As a preventative measure against the spread of mites we are keeping stock cultures (and also all other culture bottles which are used during a long period) standing in a weak soap solution. For this purpose shallow (2 inches or 5 cm high) galvanized iron trays are used. These are made to order to fit our shelves (usually 12 x 36 x 2 inches). In case any of the cultures is infected with mites the soap solution prevents their spread to adjacent cultures and keeps the infection under control. Some of our trays have been in use for over five years without any sign of wear. The initial cost for trays, therefore, is spread over a long period. Mites can also be controlled effectively by avoiding accumulation of old culture bottles and by wiping frequently, shelves and tables, with carbon tetrachloride or kerosene. (Copied from DIS 2:61).

Gottschewski, G. Control of mites. Beim Auftrreten von Milben werden Kulturflaschen und Abstellregale regelmässig mit einer 5% igen Sagrotan-Lösung abgewaschen. Ausserdem werden die vermilbten Kulturen 2 Tage nach ihrem Ansetzen erneut in frische Flaschen umgesetzt. Die Milben bleiben fast vollständig im Futter der alten Kultur und die umgesetzen Fliegen sind Milbenfrei. Dadurch ist es gelungen, die Milben vollständig zu vertreiben.

Gowen, John W. Control of mites Three or four years ago I would have said that the control of mites was relatively simple. At the present time, however, in view of my experience of the past two years, I do not consider it quite so simple - although entirely possible. My method of control consists in using cotton-stoppered bottles and in transferring pairs as soon as they hatch. This method repeated three or four times has, in every case, freed the culture of mites. Using paper caps it does not seem possible to control mites by this technique as they are apparently able to invade the bottle by way of the space left between the neck and the cap. The place where the bottles to be freed of mites are kept must, of course, be protected against contamination from cultures carrying mites.

Kyoto Laboratory Control of mites As a preventive method against the spread of mites, several larvae and pupae in the culture are put into a sieve with fine meshes, and washed with rapid running water, After the measure, they are transferred to a fresh culture bottle.

Shipman, E.E. Ridding cultures of mites. If not too many cultures are involved the following plan might be followed. Larvae are bathed in 70% alcohol for about one minute, dipped in water, and then put on fresh food. Most of the larvae survive the treatment. The writer used this method of eliminating mites from personal stocks three years ago and has seen no mites in the stocks since that time. (University of Illinois).

Columbia University Laboratory Mites and molds For keeping down mold, we have been using 0.1% Nipagin-M with both banana and cornmeal formulae and have completely eliminated mold with no effect whatever on viability. This was determined by careful experimental counts.

For cleaning stocks of mites a piece of paper on which larvae have pupated may be completely immersed in alcohol (70%) for two minutes, dried, and placed in a clean bottle to hatch. This is much simpler and more efficacious than immersing individual flies.

Crew, F.A.E. Mites and mold The addition of Nipagin M has proved a satisfactory protection against mold.

Two attacks of mites in vial cultures (but none in stocks) have been experienced. On such occasion the parasites were eliminated by segregating affected cultures and avoiding contamination. The mites were observed to enter clean cultures through the crevices often formed by the muslin coverings then used over cotton wool stoppers. When the use of muslin was discontinued, the spread of mites was rapidly reduced. Instruments coming in contact with affected cultures were sterilized after use.

Glass, B.H. Control of mold and mites. In combatting a severe infection of mold in Drosophila cultures, it has been found helpful to hold individuals for two or three days in vials of food containing 0.2% formaldehyde, added when the food is prepared. Flies can live for several weeks in such vials, inoculated in the usual manner with yeast; but larvae do not develop after hatching; and mold growth is inhibited. These vials are also very useful for holding individuals to br mated at some future time. Before transferring to fresh food, the flies are given a bath in a watch-glass of 70% alcohol for 2 to 3 minutes; then dried on filter-paper. Flies will stand a considerable immersion in alcohol with no permanent ill effects. They may be handled readily with brush and forceps. The alcohol bath treatment is also effective in freeing flies from mites. Larvae are especially easily cleaned in this way, the mites coming off at once; whereupon the larvae may be touched on filter-paper, and transferred at once to the food, using a long-handled needle, to which they gently adhere.

Schott, R. Mites and molds. When mites appeared in our cultures last summer all shelves and incubators were washed with phenol solution, pupae were isolated and brushed free of mites. Then rapid transfer of cultures followed for several generations. All old bottles were immediately soaked in phenol solution or boiled.

To prevent mold, cover surface of media with 10% alcohol, drain off and seed with yeast.

Parker, D.R. Moldex-A as a mold inhibitor. Tests were run recently to find a substance to inhibit the growth of mold. The compounds tried out were Moldex-A, Nipagin-M, and Nipagin-T. These were added to our regular banana food in the ratio of .15 grams of anti-mold substance to 100 c.c. of food. Twenty vials were made of each of the above compounds, as well as twenty vials of plain food.

One half of the vials were inoculated heavily with mold, and the other half left uninoculated. One pair of flies was placed in each vial. Moldex-A was the most efficient in the prevention of mold. However, in the uninoculated series, the Moldex vials gave a slightly lower yield of flies than did the plain food. Egg counts were then run to see the possible effect that Moldex might have on hatchability. Out of approximately 3000 eggs, 98.7% reached the adult stage. This is about 7% higher than the usual hatch on plain food at a cost of about $1.50 per pound. (Copied from DIS-4: 65).

Shipman, E.E. Mold Preventatives (Preservatives). Th. Goldschmidt Corporation, 147 Waverly Place, New York City, New York, has several different preservatives which would probably serve to prevent mold. Nipagin M has been reported in the literature but it is chemically pure and therefore more expensive than Nipagin T, the technical grade. Nipagin M is listed at $1.00 per ounce and $8.00 per pound. Nipagin T is listed at 60 cents per ounce and $4.70 per pound. They have both been reported as being used in food cultures for Drosophila in 0.15%. In a communication from the company it is recommended that Nipagin T be used in the amount of not over 0.1% since a higher amount may prevent fermentation. The preservatives should be boiled for three minutes in the water used in making the preparation.

I have no personal experience with these preservatives but plan to test Nipagin T and Nipakombin A, another of their preservatives, as soon as possible. (University of Illinois).

Amherst Laboratory Restriction of use of Nipagin M. The use of Nipagin M to stop the growth of molds in culture bottles has been found, also, to delay the development of D. melanogaster. At 28° with 0.07 gm Nipagin to 60 cc. food, the duration of the egg-larval period, in a selected stock, was increased by more than three hours. In quantitative phenogenetic studies the use of Nipagin should, therefore, be restricted. It may be useful, however, for observing the effect of increased time of development on quantitative characters.

Miscellaneous methods

Parker, D.R. Method of carrying stocks. The early method of carrying stocks in this institution was to keep them in bottles, merely shaking them from the old one into the new one at each change, with occasional etherization and examination of them. Last year, however, we adopted a new method which seems to be more efficient. The stocks are now carried in vials, keeping one old vial and mating three new ones at each change. The four are fastened together by means of a rubber band to which is attached the tag label. The flies are etherized by means of the mass method of Altenburg.

The advantages of this system are: (1) The flies are examined at each change, and (2) by making 3 new vials the chances of loss by contamination are greatly reduced. It is possible by this method to practically rid all of the stocks of mites, provided there are no adverse conditions of temperature.

This method takes a bit more time than the older one, but it will perhaps repay the loss with better stocks. (Copied from DIS-4: 65).

Muller, H.J. Labelling of stock cultures. In place of the usual practice of Drosophila laboratories of pasting a label on each stock culture and writing the name of the stock anew at each transfer, I have for many years found it much quicker and less subject to error, if the designation of the stock is written once for all in ink or India ink on both sides of a cardboard tag which is affixed through its string to a rubber band that passes around the neck of the culture vessel. This tag is transferred to the new vessel when the flies are transferred, and it is best to have a separate tag for each culture vessel. (Copied from DIS-3: 52).

Hoover, Margaret E. Maintenance of stocks. Drosophila stock bottles are kept at Cold Spring Harbor in galvanized tin trays filled with soap solution to prevent spreading of mites, and are placed on wooden shelves constructed for this purpose. These shelves measure 36 x 11-1/4 inches. Four bottles can be placed in a row the width of the shelf and about fourteen bottles lengthwise without any crowding. Our cabinets have ten shelves each from the floor to the ceiling so that one cabinet will hold 140 cultures.

Following H.J. Muller's suggestion (DIS-3, 1935) stocks are labeled by using small celluloid or heavy cardboard tags on which the labels are written; an elastic band is looped through a hole punched in each tag and slipped over the neck of the bottle. With the transfer of cultures, the tags are dipped in carbon titrachloride to prevent the spread of mites and transferred to a new bottle. Heading each row of four bottles on the shelves is a labeled celluloid tag hooked over the end of the tin tray by a card holder. The stocks are arranged on the shelves according to the listing in DIS.

Stocks are kept in 1/4 pint milk bottles. Paper milk caps with a flap are used as stoppers. Experience indicates that virilis stocks go better in small containers.

Muller, H.J. Supplying vials with paper. When numerous small vials have to be handled it is time consuming to prepare and insert paper for each one, although the presence of paper is helpful. For this purpose it is convenient to use white confetti, which can be purchased already prepared in considerable quantities. This is sifted between the fingers into the cultures en masse, as they stand still uncovered after having been seeded with yeast. (Copied from DIS-3: 52).

Brierley, Jean Method for handling vials in transfers of Active flies. I have found 30 by 100 mm. vials well suited to single pair matings. In my work it is necessary to transfer these pairs to fresh vials frequently, without etherization. The size of the vials makes it hard to hold their mouths in exact apposition during the transfer. To eliminate the chance of their slipping, I use a c-shaped metal band about 15 mm. wide, which slips over the end of the vial, protruding half its width beyond the mouth. The other vial fits into this half of the ring, and the two vials are held firmly together. Any metal will do, as long as it is fairly thin and quite flexible. It must be elastic enough to cling closely to the glass and adjust itself to the variations in sizes of vials. (University of Michigan).

Kaiser, S. Transfer of flies. We have obtained our best results in transfers by inserting into the food, cones made of towelling paper. Etherized flies come to in these cones without getting wet or dirty. The larvae creep up on the paper and pupate on it.

Stern, Curt Feeding flies kept temporarily in vials. Food (water-molasses-agar, filled into Petri-dishes, before cornmeal is added to the mixture used for bottles) is placed on a parallelogram-shaped piece of cardboard (size 42 x 25 mm. altitude 20 mm., thickness 1 mm.). These cardboard pieces are cheaper than paper spoons and are ordered per 5000 or 10,000 from stationery stores. The shape of the piece eliminates the danger of crushing flies (method suggested in 1931 by Dr. F. Koller, then in Berlin-Dahlem).

Oliver, C.P. Protection of cultures from mice. Mice have made necessary the use of a substitute for cotton plugs for cultures which are left outside a closed chamber. Paper milk bottle caps make a good substitute for cotton if milk bottles are used. Small perforations made with a dissecting needle allow sufficient passage of air. If pull-tab caps are used, the caps can be removed without damage to most of them. Dry heat is the best sterilizer.

One pound coffee cans may be used to protect cultures which are mated in shell vials. A can holds 17-19 vials, and a second can inverted over the tops of the vials allows enough air to circulate and yet protects the cultures. The cans are also of convenient size for use with some matings. Groups of similar matings can be placed in one or more of the cans and only one vial of each group needs marking.

L'Heritier, Philippe and Georges Teissier Carbon dioxide as a fly anesthetic.

In this laboratory we have found it very practical in some cases to use as a Drosophila anesthetic a stream of carbon dioxide such as can be obtained very easily from an ordinary bottle of liquefied gas. The flies fall down as soon as they are reached by the gas and keep perfectly still as long as the concentration of CO_2 is high enough. When transferred in a usual atmosphere, they wake up in less than thirty seconds and do not seem to have been injured in the least by the treatment. This technique is extremely practical when you have to transfer quickly and safely a grat number of flies from one container into another one. Formerly devised for Drosophila populations, it has been recently extended with success to the handling of several kinds of insects and of little marine crustaceans.

L'Heritier, Philippe and Georges Teissier A method for drowning and preserving the flies hatched from a cake of food.

The cake of food is set in an open vial which is hung by two small wires in a bigger bottle containing about three cms. of the following solution: mercury dioxide 1%, Nekal 0.5%. Nekal is used to lessen the surface tension in order that the flies get drowned as soon as they are in contact with the liquid. Nekal can be purchased from the "I.G. Farben Industrie, Germany". Such a preservative solution does not give rise to any injuring vapor. Once a day or more often if desired, it is easy to shake the bottle and drown all the flies which have hatched since the last shaking. The total yield of the cake of food can thus be obtained without interference of a second generation.

Hoover, Margaret E. Transportation of Drosophila cultures.

For mailing Drosophila, we have been using 7 x 2 cm. shell vials. The vials contain a small amount of the usual cornmeal-agar prepared food, innoculated with yeast, and a strip of paper is inserted to prevent the food from running onto the sides of the vial. From one to seven vials will easily fit into corrugated paper boxes 8 1/2 x 6 3/4 x 6 3/4 cm. If the vials are wrapped in paper and tightly packed on all sides by cotton, there is no danger of breakage. We have found this to be a very satisfactory method for transporting stocks. Both Drosophila melanogaster and virilis cultures have been satisfactorily shipped as far as Japan. The mailing costs are low. The packages may be sent third class in the United States and as small packets or samples to foreign countries. A full package will usually not weigh in excess of six ounces. (Copied from DIS-3: 51).

Parker, D.R. Washing bottles and trays. Old food is removed by means of a jet of water from a reducing nipple. The bottles are then soaked at least an hour, in order to soften the pupa cases, in a hot soap solution to which a small amount of phenol is added. The bottles are brushed with standard milk bottle brushes, powered by a 1/4 H.P. electric motor. The vials are washed with a Fuller white bottle brush (no. 18423 Fuller Brush Co., $2.40 doz.) on the same motor. Then they are rinsed thoroughly and allowed to drain and dry before being used. "Tins" and wooden trays are washed in the phenol solution each time they are used. This procedure is very important in the control of mites.

WORK SHEETS

1. External Structure of Drosophila (C.B. Bridges) (See DIS-3:55)

2. Reference Map of the Salivary Chromosomes of Drosophila melanogaster. (Reprint from Journal of Heredity Vol. 26, No. 2, February 1935; see also DIS-3: 55). A limited number of copies of the map are available for research purposes.

3. Side view of wild type for detailed study of bristles.

4. Dorsal view of head and thorax for detailed study of bristles.

5. Wings of Drosophila virilis

6. Diagrams of normal ovaries and testes. (Used by G. A. Lebedeff for detailed study of intersexes).

Research Notes

Auerbach, C. Studies on egg shape and fecundity in D. funebris.

The mutant spheroidal was investigated. The eggs are short and roundish, the filaments are short and stiff and do not spread out as in normal eggs. At the same time fecundity is very low. Hatchability appears to be good. Spheroidal segregates as a simple Mendelian autosomal recessive. Inverstigations have been started to study its effects more in detail, with a special view as to the nature of the connection between the morphological character "shape of the egg" and the physiological one "low fecundity."

G. Bonnier and M. Nordenskiold. Attached X's.

Selections have been performed in order to get different percentages of homozygosis at the yellow-locus. In this way a high and a low line have been established. In 466 cultures of the high line this percentage is 22.80 ± 0.34 and in 474 cultures of the low line the percentage is 17.72 ± 0.24. This selection has at the same time produced a selection of the rate of detachments. Within the same cultures there has been a total of 3 detachments in the high line, and 858 detachments in the low line. Investigations concerning the genetical causes are now under hand.

Brehme, Katherine S. A method for counting the larval instars in mutant stocks of Drosophila.

It has been suggested that a fourth larval instar might explain the increase in duration of the larval period of certain mutant types beyond that of the wild type, in D. melanogaster. Such mutants include heterozygous Mw, with a larval period about two days longer that that of + sib at 25° C, and giant, which pupates three to five days later than non-giant sib at 25°. The following method has been devised for ascertaining the number of larval instars.

A thin layer of Pearl's S 101 medium is placed in a three-inch Petri dish and sown with one drop of yeast suspension. Ten eggs are cultured in each dish at 25°. When all larvae in the culture have pupated, the pupae are removed to paper spoons containing a 2% solution of agar (for moisture supply) and are kept in cotton stoppered shell vials until the adults emerge, when the adult phenotypes can be recorded. After removal of pupae, each dish is examined on a white background under the binocular (9 x ocular, 2.3 x objective); the molted mouth armatures are easily seen on this almost transparent medium. A piece of graph paper under the dish affords a means of orientation, so that the entire dish can be thoroughly examined. In this way it is possible to collect all molted mouthparts. The absence of a set of discarded mouthparts, in addition to the first and second instar sets and those in the pupa cases, proves the absence of an extra instar. Any mouthparts which might be overlooked in this inspection can be recovered by melting the medium and filtering through a small cone of filter paper, on which the mouthparts can be located under the binocular. The writer has used this method to demonstrate that heterozygous Mw has three instars only. The case of giant is going investigated, with substitution of finely strained banana agar for the S 101 medium.

Cochrane, Flora Eye colors of D. pseudo-obscura. A histological study of wildtype and seven eye color mutants of D. pseudo-obscura at various stages in development has been made.

Sepia affects pigment during the late phase of pupal development and during adult life. The influence is a chemical one due to which all of the eye pigment eventually becomes yellow and brown. The actual amount of pigment is probably not reduced.

Eosin suppresses the formation of part of the pigment granules throughout pupal development but does not appear to influence them chemically. Purple affects the rate of production of red pigment. Purple^2 retards the formation of red so little as to make purple^2 almost indistinguishable from wildtype; only in combination with vermilion or orange is the effect of purple^2 obvious. Purple^3 retards the production of red considerably, and purple^1 to such an extent that few red granules are present at emergence but many appear in older flies. Vermilion and orange suppress the entire early phase of pigment development but allow the late phase to proceed as in wildtype.

In a culture resulting from a pair mating of eosin (w^e) flies three ♂♂ were found which had slightly pigmented eyes. This color which was found to be allelmorphic to w^e was called buff (w^{bf}). By mating buff ♂♂ to wildtype ♀♀ buff ♀♀ were obtained in the F_3. The eyes of buff contain more pigment than those of the ♂♂.

Crew, F.A.E. and R. Lamy. The Px Inversion in pseudo-obscura. The sex-linked character, Plexus, which much resembles the autosomal character called smoky by Dobzhansky, has been found to be associated with a very small inversion between y and w, and is not necessarily connected with the larger inversions on the X-chromosome described by other writers (Dobzhansky, Tan, Koller) though the larger inversion existed originally in the px stock. It is uncertain whether the mozaics that occur in px matings are connected with the large inversions or with the Px inversion proper.

The crossing-over data on which a former hypothesis of the size of the Px inversion was based, was considered on the assumption that the order of the genes was y v sn (Lancefield). It appears that the order is y sn v, and hence crossovers regarded as doubles were really singles. In heterozygous Px the crossing-over values observed are:

y Px	.15	12692
Px w	.15	"
y sn	.6	2943
sn v	.2	"
v dy	.1	"

Crew, F.A.E. and R. Lamy. Notes on Nomenclature. It would seem that the naming of mutations is a task that can no longer be safely left to the individual. It is possible that the time has now arrived when a Nomenclature Commettee should be instituted. These remarks are provoked by the fact that in a recent paper (Genetics, 21, 1) Mr. Tan arbitrarily renames our "short$_4$", calling it "incomplete"; thus brushing aside the reasons for our choice.

It has been suggested that mutations which are phenotypically indistinguishable should be described by the same name, the numbers of the chromosomes carrying their genes being added as distinguishing marks. To us, at least, this seemed to be a reasonable and useful practice. Our "short" which was phenotypically indistinguishable from Lancefield's sex-linked short, was named, therefore, "short$_4$".

We had no hesitation in accepting Dr. Dobžhansky's and Mr. Tan's re-numbering of the linkage groups, since the numbering previously employed by ourselves was purely chronological and could only be regarded as temporary. But to rename short$_4$ is quite a different matter, for there seems no valid reason for doing so.

De Marinis, Frank and A. H. Hersh. Temperature effect on bar-eyed mosaics. White mosaics of bar-eyed flies raised at 20° C. conform to the relative growth function in regard to the quantative relation between the red and white facets, as was shown previously for similar mosaics raised at 25° (Hersh, 1934 Science, 80:547). There is practically no shift in the value of a, but b is much lower at 20° than at 25°. For further comparison a series of similar mosaics at 30° is being collected.

Howland, R. B., E. Glancy and B. Sonnenblick. Development of larval wing-thoracic discs on implantation in D. melanogaster. Implants of the dorsal mesothoracic disc from mature larvae of various mutant types have been made in wild type hosts of the same age. The donors used were ey^D/ci^D, D3, ss, f B, sn^3, sc, y sc, and an extreme form of H^2. The disc gives rise to thewing, half-thorax and half-scutellum. These regions are distinct in the implant. It is possible to identify the thoracic and scutellar bristles in favorable cases. Development of the wing is normal except for the absence of venation. Data so far obtained indicate that all mutant discs used develop autonomously without producing any phenotypic modification of the host. However, even in wild type control implants a number of bristles, especially the larger ones, are usually bent and otherwise distorted so that they resemble forked and singed. Since the implant develops in an inverted position, these abnormalities are in all probability due to compression of the bristles during development. This interferes with interpretation of results obtained on implantation of wild type and mutant discs in forked and singed hosts. At the present time, experiments on the implantation of wild type and mutant discs in mutant hosts are under way.

Howland, R. B. and J. W. Jailer. Microcautery of posterior pole-plasm and germ-cell determinants in D. melanogaster.

Timed eggs of D. melanogaster in which the cleavage nuclei have not yet reached the posterior pole-plasm were dechorionated and aligned on thin slabs of agar. The posterior region, subjected to heat from the point of an electromicrocauter, was burned to an extent varying from 1/8th to 1/6th of the entire egg. The living portion of the eggs constricts off gradually from the burned mass. A blastoderm is laid around the entire living portion. No germ cells form at the posterior pole. The operated embryos undergo the typical proctodeal-amniotic invagination, but no pole cells are carried inward in this region. From a large number of embryos but one adult hatched. This fly, a male, was crossed to four virgin females. All these females laid sterile eggs. In section of the small mass of testicular tissue of the male no spermatogonial cells or sperms were seen. It is apparent that there is in the prelocalized posterior egg region, either in the pole-plasm or the granular inclusions or both, the essential materials for germ cell formation.

Howland, R. B., E. Glancy and B. Sonnenblick. Interspecific transplantation of wild type and vermilion eye discs in Drosophila.

Interspecific implantations of wild type and vermilion eye discs were made within and between four species of Drosophila. The stocks used were D. melanogaster (Woodbury + and v^2) from Washington Square College: D. simulans (+ and v) and D. pseudo-obscura, Texas Race A (+ and y sn v) from Pasadena; and D. virilis (American + and v^3 mt^3) from Cold Spring Harbor. The wild type eyes develop autonomously in all cases, the color of the virilis + eye being darker (more like garnet) than any of the other three species. The diffusible v^+ substance postulated by Ephrussi and Beadly acts upon and modifies to wild type implanted vermilion eye discs of every species except that of D. virilis (v^3 mt^3). In this case, the eye color though intermediate with respect to the virilis + implant, matches that of the + implants of the other species. In further experiments, darker allelmorphs of v^3 are being used.

Just G. and Steiniger, F. Natural selection in D. melanogaster (normal-winged and vestigial) on the isle Greifswalder Oie.

In the spring of 1935 an investigation was entered on the isle cited to determine the value of selection under natural isle conditions in vestigial and normal-winged D. melanogaster, both put out experimentally on the isle. The investigation was continued in 1936 and will be also continued in 1937.

Lapedies, Daniel L. The effect of ci^D upon the facet number of Bar eye in D. melanogaster.

Isogenic Bar females were mated with bt^D/ci^D males at 25° c. In the F_1 the facet numbers of the males were counted with a net micrometer. The facet numbers of 48 F_1 B; $ci^D/+$ males = 48.0 ± 1.4, the facet number of 57 F_1 B; $bt^D/+$ males = 58.87 ± 1.8. The size of the F_1 female eye made facet counts impractical. While the B/+; $bt^D/+$ female eye was similar to the heterozygote B/+ female eye in shape and size, and B/+; $ci^D/+$ female eye, due to a loss of facets along the entire anterior edge of the eye, was smaller and exhibited a different shape that showed little variation.

Neuhaus, M. Sterility mutations in D. melanogaster.

In order to detect genes in the X-chromosome, nonhomologous to bobbed but homologous to the Y, the following experiment was undertaken: yellow males were X-rayed (dosage about 5000 r) and crossed to $ClB/w^e bb^1$. Bar females from F_1 were mated with $w^e bb^1$ males. Mutations homologous to bobbed and those arising in the active part of the X were obtained in F_2. Non-Bar females from F_2 were crossed to their brothers and if recessive mutations, having homologous in the Y arose in the X, then in F_3 it would be possible to obtain females showing the same mutations. Among 1136 chromosomes examined the above mutations did not occur but at the same time it was found that in some bottles (10%) of F_2 all males carrying the irradiated X-chromosome were sterile. This fact being established those males' sisters were crossed to y. v f B males, all sons from F_3 having been tested on sterility. The following table shows a part of the results obtained:

	Fertile males		Sterile males		
Stock number	y	w^e	y	w^e	y w^e
No. 6	3	1	6	8	-
No. 15	4	4	5	2	-
No. 7	4	5	7	5	1
No. 19	-	11	6	4	-
No. 20	5	8	7	9	1
No. 18	3	7	9	5	-
No. 23	1	6	6	2	-
No. 21	3	1	7	1	-
No. 4	1	4	10	5	-
No. 8	3	6	5	3	-
No. 13	3	17	20	2	-
No. 22	3	6	4	4	-
No. 2	-	11	7	-	-

The above data allow us to conclude that under the influence of X-rays sterility mutations scattered along the whole length of the X-chromosome may arise.

Spencer, W. P. Life history and control of laboratory mites.

See DIS-6;67-68. The following observations on the life history of the parasitic laboratory mite are of interest in connection with the problem of control. On Sept. 16 one D. repleta carrying a single individual of the parasitic mite was placed in a shell vial with banana agar culture medium. By Sept. 24 the mite, fully grown, was crawling in the culture medium. By Sept. 25 a number of young mites were seen in the culture thus indicating that the mite reproduces parthenogeneically (mating of the immature parasitic stage has never been observed and probably does not occur). On Sept. 26 there were about 100 young mites in the culture vial. These moved rather rapidly over the surface of the culture medium or slowly if the legs became immersed in it. There was no tendency for these mites to wander far from the surface of the culture up the sides of the vial. On Sept. 27 these mites had grown to a size larger than the migratory stage and one pair was observed mating. On Sept. 28 several mating pairs were present and a single specimen of the migratory stage was seen. These observations indicate a life cycle in which parthenogenesis produces both males and females. Thus a culture or a laboratory may become infected from a single mite of the immature migratory or parasitic stage.

Sources of infection. A laboratory entirely free of parasitic mites may become infected from the following sources:

(a) Cultures received from other laboratories. Often only a few mites will be present in such cultures when first received and will not be detected. Always assume that they are present.

(b) Wild flies. Among 1232 flies taken in traps in Pasadena and several nearby points in October, November, and December 1936 five mite-infested flies were found. The collections included 13 species and mites were found on individuals of simulans, pseudo-obscura, and an undescribed species related to hydei. Also in a collection of perhaps 150 hydei 1 mite-infested fly was found. Unless wild flies are being collected in large numbers this source of infection is not as important as (a).

(c) Other insects. I have twice taken wild specimens of Eucoila, (a parasitoid wasp which preys on Drosophila) which were mite-infested.

Methods of control. Follow points outlined fully in DIS-6:67-68. The following points should be particularly emphasized. (a) Watch for all stages of the mite as a routine when examing cultures. (b) During infestations keep all cultures in Lysol solution 1:200. (c) Repeatedly clean table tops, all instruments including etherizer, and incubator shelves. (d) Heat is the surest method of killing all stages of the mite in discarded cultures, and it is also the most economical. (e) Rapid transfer of stocks. (f) Use of very tight cotton plugs following Gowen's suggestion (DIS-6:69).

Technical Notes

Altenburg, E. Stocks. Most stocks can be kept in vials for 1-2 months without transfer at a temperature of 18°-20° C. (Vestigial, however, becomes sterile at a low temperature). This is an economical method of keeping stocks, particularly stocks that are not greatly in demand. It is also a good way to keep stocks in duplicate, or during periods of protracted absence of the investigator, as during vacation time.

Demerec, M. Control of mites. As a preventive measure against the spread of mites we are keeping our culture bottles in galvanized iron pans made to fit our shelves. These pans were originally filled with a weak cresote solution which was, because of its odor and fast sedimentation, later substituted by a strong soap solution. The object of the solution is to prevent the spread of mites from one culture into another and thus eliminate the source of the infection. Soap solution, however, was found inadequate for mite control. After investigating several possibilities it has been found that light oil is a good medium for this purpose. Upon request, The Standard Oil Co. of New York supplied samples of light oils, non-volative, non-inflammable, and odorless, out of which the type called Mineral Seal Oil was selected. It has been in use since October 1936, with very satisfactory results. This oil costs 35 cents per gallon in 5 gallon lots, or 17 cents per gallon in barrel lots. To cover bottoms of seventy 12x36x2 inch pans about 30 gallons of oil were used.

Marshak, A. Salivary chromosome slides.

Examination of slides prepared by the technique previously described and mounted in carmine-saturated glycerine, have been reexamined a year and four months after their preparation. The chromosomes are still well preserved with the bands well defined and with no apparent change since the first examination.

Ono, H. Induction of mutations by electric current.

Larvae of D. virilis were placed in 24 mm^2 paraffin canal filled by ca. 1/100 N KCl solution. Direct current of 6 M.A. was transmitted through the canal for 7 minutes. Out of 162 F_1 flies raised from these treated larvae 37 were mutants (all of wing deformity). Under normal conditions this stock gave about one per cent wing deformed mutations.

Shapiro, N. The method of studying the process of mutation in a limited region of the chromosome.

The investigators usually when studying mutation induced by X-rays use either the ClB method, or the method of attached X-chromosomes. In both cases mutations arising along the whole lenght of the X-chromosome are picked up. The solution of many important problems of genetics requires sometimes the study of the mutation process in a limited region of the chromosome. This may give the following possibilities: (1) to follow all the variety of mutation in a small region of the chromosome. (2) to establish the number of loci present in the region capable to mutate. The latter will enable us to estimate the minimum number of genes not only in the region studied but in the whole chromosome set. Using the current genetical method and wishing to isolate among the mutations found only those which are located in a definite region we are obliged to use the crossing-over method. The latter offers many technical difficulties and sometimes is even not valid to solve the problem (when the mutation is associated with an inversion). We propose, therefore, to modify somewhat the ClB or attached X methods for cases when the investigator wants to study the process of mutation in a limited region of the chromosome.

We give two examples for the study of the left end of the X-chromosome in order to illustrate the modification we suggest.

(1) Yellow males are X-rayed and mated to attached-X-females, carrying a deletion. F_1 males with the deletion are selected and mated individually with ♀♀ XX. In case a lethal arose during treatment in the region of the left end covered by the deletion, the progeny of such a cross would give only males carrying the deletion. No yellow males will appear.

We are able, therefore, to pick up automatically only those lethals which are located on the extreme left end of the X-chromosome.

The same method can hold for the study of visible mutations. It is necessary for this purpose to examine both yellow and non-yellow males in the offspring of the cross.

(2) A yellow male is X-rayed and mated to ClB female carrying homozygous yellow and a deletion. F_1 Bar females with a deletion are selected and crossed individually to yellow males. In case a lethal arose in the X-chromosome during treatment in a region not covered by the deletion, no males appear in such a cross; if the lethal arose in the extreme left end of the X-chromosome only males carrying the deletion will survive.

The same principle as that given above will hold true for any region of the X-chromosome; it is only necessary to have a special duplication, producing no lethal or sterile effect on males or females.

The method for the study of the mutation process in a limited region of one of the autosomes is somewhat different. While for the study of mutation in separate regions of the X-chromosome it was necessary to have duplications, in the case of autosomes it is rational to have deficiencies. Let us consider the case of Np deficiency (Bridges, SKoog and Ju-Chi-Li, Genet. 1936). A heterozygous Cy male is X-rayed and mated to Cy/L females. L/+ (or Cy/+) males are selected and mated to females carrying in one of the chromosomes a deficiency Np. The lack of Np flies and the presence of Np/L (or Np/Cy) flies will indicate that a lethal arose in the region Np. All matings ought to be individual. The treated male must have only one normal chromosome which is to be analyzed. About 15 chromosomes are to examined from one X-rayed male, as it is possible that the chromosome under investigation contained originally a lethal in the region of the deficiency. In the latter case all the 15 cultures from one male will show the presence of a lethal.

Spencer, W.P. A new technique for growing Drosophila.

The method of Drosophila culture about to be described is not simply an improvement in current methods but rather a new departure so different that for the present in some laboratories it may be applied gingerly at first and only to certain special problems. The method consists in treating eggs, larvae, pupae and adults as distinct organisms in so far as their culture conditions are concerned. It favors spending as much time on the quantitative and qualitative study of eggs and larvae as has in the past been given to the study of the adult. This method is made possible by the discovery that the Drosophila female will readily deposit her eggs through a fine mesh of silk upon the proper medium. This fact makes it possible to standardize methods of culture all along the line and check each step by the use of adequate controls. With the wealth of material already at hand in the form of genetic tools and the new technical method of handling Drosophila it now becomes possible to plan and carry out experiments with the precision comparable to that employed in the chemical or physical laboratory.

The Drosophila adult (or adults) are confined in a tiny cage, the design of which will no doubt change with further experiment. The cage in current use consists of a metal gas-hose ferrule 7/8 inch in diameter and 1/2 inch high. Over one end of this ring is stretched tightly a piece of black silk bobbinette trade No. 400 for large Drosophila species and No. 418 for small species such as melanogaster. This silk net is held in place by a small rubber band. Into the smaller end of the ring fits an ordinary cork of such a size that it projects part way into the ring and cut off so that about a quarter of an inch extends out of the ring. The adult flies, either for stocks or experimental cultures, are kept in this tiny chamber. The label goes on a small slip of library card which fits at one side of the cork. This ensures plenty of air entering the chamber. The present system of labeling stock bottles with long symbols will be discarded in favor of the less cumbersome DIS stock list number which may be written on the cardboard slip or on the end of the cork. For experimental cultures generally one female will be used per chamber as the method makes possible now quantitative study of daily and hourly output. For securing eggs the cage is placed directly on the medium on which the eggs are to be laid. This medium will presumably consist for the present of yeast enriched cornmeal agar or banana agar with a little animal charcoal added for contrast. The medium is poured into small water-proofed plates of a size conveniently examined on the stage of the binocular. Yeasting is done by the method described by Muller of spraying with a fine yeast suspension from an atomizer. The yeasting should probably not be done more than an hour before the plate is to be used, if the subsequent exposure of the plate to oviposition lasts for several hours. The best time may be checked for each species. As many experimental cages as will fit conveniently on the agar plate may be placed there. At the time of placing the cages a key to their position is written on the edge of the plate, together with any other pertinent notes on the experiment. An inverted paper plate covers the apparatus and may be held in place by paper clips or two rubber bands at right angles. With this precaution there is even less danger of contamination than in cotton plug bottles. First the wild flies are excluded from the plate and second the investigator is only interested in the spot under each cage which is effectively shielded by the cage itself from contamination. After a given time the cages are lifted off and quickly transferred to a fresh agar plate. Apparatus may easily be designed which will remove agar plates and set fresh ones in place mechanically without disturbing greatly the flies enclosed in the cages.

The agar plate on which oviposition has occurred is then put under the binocular or examined with a hand lens and if the experiment calls for it the egg output of each female is counted. If the experiment involves the raising of larvae, pupae, or adults a given number of eggs will be cut off with a scalpel or other instrument and these eggs placed in bottles or vials until other better containers are designed. These containers will contain cotton or other porous material soaked in a given amount of yeast suspension known to be more than the minimum amount required for the optimum development of a number of larvae equal to that of the number of eggs used. These vial cultures will, in many experiments, contain eggs all of which have been laid within a few hours. The conditions of development will be standard. The agar plates containing the remainder of the eggs will be placed at the same temperature as the vials and the remaining eggs or portions of them used as an index of hatching. Even though larvae hatch and crawl from spot to spot they do not move unhatched eggs for two days or more, nor is there appreciable shift of egg cases for the first two days. These points may be checked as I have already done repeatedly. With the proper design of larval culture dish the eggs added to the dish may be checked directly for hatching thus eliminating the dangers of fair sampling. In many if not most experiments the investigator will examine the agar plate or larval culture throughout development, observing the larval behavior and genetic differences in cases where visible. Routinely egg and larval mutants will be picked up as are adult mutants today. All larvae (except in very exceptional genetic set-ups) will have pupated in a given dish before the first adults emerge and the handling of adults may be much as in the old technique.

Stocks will consist of adult stocks and larval stocks. The adults of the two sexes will be kept separately in cages identical with or larger than the one described above, sitting on enriched media which have been treated to prevent mold and bacterial growth and to inhibit oviposition. They will be kept at temperatures so low that the same set of adult flies may be held for a long period of time (possibly three months). The length of life is prolonged both by the very low temperature (12°±) and rich feeding accompanied by low metabolism and lack of expenditure through egg laying. Always moreover, a parallel set of larval stocks will be kept in vials on yeast. Possibly ingredients may be added to these larval cultures to inhibit growth without being harmful. Certainly low temperature may be employed here. The details as to the exact form in which larval and adult stocks are kept will probably vary from laboratory to laboratory.

Mite infections can never be serious since the life cycle of the mite is broken up by the use of two sets of stocks. Suppose a mite infection to occur among adult stocks. The life cycle of the mite would be broken up by several mass transfers of these stocks to fresh media. This would be the work of a few minutes as the cages may be fixed to holders, allowing a slight movement of the cage on the holder (so that no cage would ever accidentally be held off the medium) and a hundred cages lifted from the old agar plate to a new one at once. If a mite infection broke out in the larval stocks, first new larval stocks would be established from the adult breeders and then old ones discarded en masse. However, the real reason that mites have flourished in Drosophila laboratories is because the stock keeper could not see what was going on in a half-pint milk bottle and generally did not trouble to see what occurred in a culture vial. Egg and larval stages will be under the microscope as frequently as adults and mites will have no chance. They might, however, prove interesting objects for physiological or genetic research when we no longer fear their depredations.

No doubt improvements will be made in the technique here suggested, and suggestions will be welcomed. The method offers too many advantages to be held out of use while all details are perfected. I have only recently developed the method above described, using such suggestions as have already appeared in DIS and the literature in regard to yeasting, charcoal, growing larvae in yeast. It works for studies in life histories, egg counts, egg hatch counts, examination and comparison of eggs and larvae of different species en masse, discovery of egg laying rhythms in different species, time at which hatching per cent of eggs begins to lower (probably due to sperm supply running out) when females mated to males are then put alone into oviposition cages. The value in the method lies in the opportunity to check every factor at every point in the life history of the fly. Exact quantitative checks and controls may be employed and it is bound to lead to a toning up of our technical methods at all points. For instance if one questions the size of the breeding cage either as regards volume or area exposed to food, the comparison is immediately open to exact quantitative experiment. I have checked food exposure areas and two types of silk nets and done the two things in the same set of experiments. The introduction of this method makes relatively easy the investigation of many problems in the behavior, physiology, and genetics of Drosophila hitherto unapproachable because of the time involved and the impossibility of standard conditions in the experiment. It is to be hoped that many laboratories will give it a trial.

During the course of developing the apparatus I have kept in mind the matter of expense of equipment and labor involved in the procedures. As soon as certain problems on size of cage, etc. have been more thoroughly tested it should be possible to have breeding cages of perhaps several sizes and designs made up to sell at low cost. This is more likely to happen if those interested go directly to the manufacturer rather than putting the matter in the hands of supply houses. In the past I have corresponded with the Aluminum Company of America relative to aluminum shipping vials which could be used to send stocks by airmail letter with no additional postage and know that vials could be secured and made over for a little more than two cents each. Cellophane will probably play a part in some cage designs. Whatever material is used, cages must be light in weight for best results. Otherwise an exudate is pressed from the agar plate.

Cost of material used in the above tests

1 yd. 72 inch width, of black silk bobbinette No. 400		$3.00
1 yd. 72 inch width, of black silk bobbinette No. 418		$4.00
(A yard will make up 1000 or more cages)		
Metal gas-hose ferrule, 7/8 inch by 1/2 inch per 100		$2.50
Water-proofed paper plates, 5 inch diameter	18	.05
Atomizer for spraying yeast		.85

There is no doubt but that in terms of research production the cost both in money and in time will be far below that now expended. (See also Science 85:298. 1937).

Stalker, H.D. Mounting and handling dried specimens pf Drosophilinae.

For cementing Drosophilinae and other small insects to cardboard points we have found the following much superior to mucilage, shellac, etc.

10 cc. glacial acetic acid, equal volume of powdered gelatin, (Knox).

The gelatin is dissolved in the cold acid and the cement is ready for use.

For immediate relaxation of dried specimens, household ammonia, applied a drop at a time until the insect is thoroughly wet, will cause complete relaxation in two or three minutes. If, in the case of insects on points, the excess ammonia dissolves the cement, the specimen is limp enough to be remounted with safety. When the specimen dries out it will be found to be unharmed by the treatment.

Williams, C.R. Collecting eggs and larvae.

Size number 47 ramekins, made by American Lace Paper Co., Milwaukee, Wis., which have been previously dipped in paraffin are used. About six mm. of banana-agar-yeast food is placed in the ramekin. About eight pairs of mated flies are placed in a 3x9 1/2 cm. empty shell vial. The ramekin containing the food is then placed over the shell vial; the whole is inverted so that the food is ventral to the flies. They are then put away in a dark container for the desired egg-laying period, after which the ramekin is removed and capped with an ordinary milk bottle cap. With this method the ramekin can be observed under the binocular with ease. The larvae should not be left in ramekins more than fifty hours as the food dries up after this length of time. The flies in the shell vials may be immediately used in another egg-laying period or else returned to vials containing food.

Research Notes

Blanc, R. and Child, G. P. Reversal of dominance in the dumpy locus in D. melanogaster.

Larvae and pupae of heterozygous dumpy flies were subjected to 36.5° C. for 12 hours. A large percentage of the flies exposed to the heat treatment beginning at 12-16 hours of pupal life showed truncated wings. These varied from nearly normal through a simulation of the effect of the oblique allele of dp to an effect greater than that of homozygous dp. The treatment producing the greatest effect was that begun at 13-14 hours of pupal life. Females showed a less pronounced effect than males both as to number and degree of the truncates. The period of greatest effect was one to two hours earlier in females than in males. Vortex, part of the phenotypic manifestation of dp, showed an earlier temperature effect period, at 6-10 hours of pupal life. This reversal of dominance has been observed with respect to other genes, such as cv, f, c, px. The experiments are being continued with the use of a dumpy stock and a wild stock which are isogenic to each other with the exception of the region of the dp locus.

Brahme, Katherine S. A larval character in D. melanogaster.

In a study of the attached-X *yw*/+ stock, it has been found that the mouth armature of homozygous yellow larvae is characterized by a lighter color than that of non-yellow. This can be diagnosed with certainty in the living larva at all stages from hatching to pupation. The color is lightest in the first instar larvae, where the whole armature is very light brown, almost golden; wild type armature at this time is very dark brown. In the second instar, the armature is somewhat darker than in the first but all regions of the armature are distinguishable from wild type. In the third instar (about 70 hours until pupation at 25° C) the mouth hooks are as dark as those of the wild type; the middle region of the armature is somewhat lighter; the posterior part of the armature is light brown, in contrast to the very dark brown of the wild type. Separation of *y w* XXY females from wild type males and *y w* triple-X females was made by means of mouth armature color, and when checked against the colorless condition of the homozygous white Malpighian tubes, was found to be a perfectly accurate means of classification. That the mouth armature color is dependent upon the yellow gene and not the white was determined by examination of white and of yellow stocks. This character has been observed independently by N. Kaliss in another stock.

Brehme, Katherine S. Effects of the triple-X condition in D. melanogaster.

In a study of the giant larva, using the gt bb^{11}/gt w^a stock, a high pupal and first instar larval mortality was observed, as well as a high frequency of zygotes whose pupation was retarded, as expected for giant, but which did not form large (giant) pupa cases. In order to separate the effects of the triple-X condition from those of the giant, a study of larvae and pupae of y w/+ stock has been made. Counts based upon 350 experimental and 1000 control larvae show about 10% larval mortality, 17% pupal mortality in the attached-X stock, in per cent of total zygotes; Florida wild type controls show about 8% mortality in the larval stage, 1% in the pupal. In the y w/+ stock, 2% emerged as superfemales. Of the dead larvae, one-fourth died in the late third instar and were identified as triple-X by the color of the Malpighian tubes and mouth armature; the remaining dead larvae were all in the first instar and practically all were males and XXY females. In this stock, therefore, the lethal effect of the triple-X condition is confined almost entirely to the pupal and prepupal stages. In the gt bb^{11}/gt w^a stock, a larval mortality of 14% (almost all in the first instar) and pupal mortality of 16% were observed. Apparently the triple-X lethality is affected by the genetic environment, a larger proportion dying in the early larval period in the giant stock.

In the y w/+ stock, it was also observed that triple-X larvae do not form puparia until about 24 hours later than the mean pupation time of the males and XXY females; some XXX larvae do not form puparia until 7 days after oviposition at 25° C. Although no measurements were made, larval growth does not seem to occur during the additional days of larval life.

Barigozzi, Claudio. Study of salivary chromosomes through the ash analysis.

Spodograms of D. melanogaster salivary glands were prepared in order to determine the presence of inorganic materials in the ash. After burning at 450°-500° C the residue was easily detected in euchromomatic regions, but no ash was found in the chromocentral region. This indicates that the euchromatin is rich in inorganic matter while the heterochromatin is either poor in it or that such materials are entirely eliminated at temperatures of 450°-500° Centigrade.

Bedichek, Sarah. A spontaneous reverse mutation of $yellow^2$.

A reverse mutation of y^2 occurred spontaneously in a homozygous y^2 v f triploid stock. A single diploid female of the constitution + v f/y^2 v f was found; contamination is thus excluded.

This reverse-y^2 was not only dominant over y^2, but also over the y allels in the stocks y 99b, y 303h, and y ac sc^8 w^a 13 m. A cytological examination for chromosomal abnormalities has not yet been made.

Buchmann, W. Temperature experiments. Experiments with temperature shocks were performed in order to study the effects of these temperature shocks on the duration of the developmental stages and on the presence of modifications of D. melanogaster. My experiments, which are not yet completed, showed that temperature shocks slow development. At the same time it was found that there exists a parallelism between the hereditary and nonhereditary variability. The nature of the induced nonhereditary modification depends upon the treated developmental stages and upon the applied temperature.

Cochrane, Flora. Color of testis. Study of testis color in 20 eye-color mutant stocks of *D. pseudoobscura* showed that the amount and quality of color present in the testes is comparable to the amount and quality of the pigment deposited in the eyes during the late phase of their development. It was also found that color appears in the testes at about the time of the onset of the late phase of eye pigment development and may therefore be affected only by genes active during this period.

Crew, F.A.E. and Rowena Lamy. Mosaics in D. pseudo-obscura. Thirty-eight mosaics have been obtained. They appear to be caused by chromosome elimination. First and second cleavage mosaics show no signs of gynandromorphism. Sex-combs develop on XX legs of male mosaics and not on the XO legs of female mosaics A fertile female mosaic having an abdomen bilaterally divided into XX and XO tissue produced a high number of sterile exceptional sons, which is considered as evidence that she had incorporated in one ovary some germ cells which were XO in constitution. Vermilion in those mosaics behaves similarly to vermilion in *melanogaster* and *simulans*; that is, it appears as a wild type eye in exceptional tissue. In two female "fore-and-aft" mosaics however in which the head and thorax were XO and the abdomen XX (and v/ ≠) the eyes were vermilion. Sepia and white show autonomous development in exceptional tissue. There is some indication that sex-dimorphic characters are expressed according to the sex of the mosaic and not according to the constitution of the tissue.

Dubinin, N. P., N.N. Sokolov, G.G. Tiniakov and V.V. Sacharov. Unilateral chromosome conjugation in the salivary gland cells of Drosophila.

In our work published in 1935 (Biologicheskij jhurnal, vol. 4, No. 1, Russian) we wrote about unilateral chromosome conjugation in the salivary gland cells, basing our work on an analysis of heterozygous aberrations. However, we have come to the conclusion that the material used cannot prove our point, because the homologous chromosomes are twisted about each other. The problem of unilateral chromosome conjugation remains, therefore, unsolved.

Dubinin, N.P., N.N. Sokolov and G.G. Tiniakov. Crossing over between the genes "yellow" "achaete" and "scute".

Crossing-over between y ac and sc in females of the composition y ac^3 sc^1 w f/+ has been studied. The females were in all instances heterozygous for the C^2R-Cy^2L.l. inversion and in half of the cases they were at the same time heterozygous for the C^3R-1-C^3L inversion. The inversions were introduced in order to increase crossing over at the left end of the X-chromosome. Crossing-over between ac and sc was obtained. A total of 75578 flies was investigated, and among them four y ac^3 sc^+ flies and one y^+ ac^+ sc^1 individual were found. Genetic analysis of crossovers excludes the possibility of contamination. One y ac^+ sc^+ fly was found in the experiment. Crossing-over between yellow and achaete was therefore suspected, but the fly died and due to the impossibility to test it further, this problem remains unsolved. The experiment showed a general increase of crossing over at the left end of the X-chromosome. Crossing-over between yellow and white amounted to 3.7 per cent (n = 32548).

If the supposition is correct that ac and sc are adjacent, then crossing over between two adjacent genes has been proved for the first time. Under the conditions described above this crossing over occurs with a definite and relatively high frequency.

Dubinin, N.P., N.N. Sokolov and G.G. Tiniakov. D. simulans from Adzharistan.

According to the data of O. Duda communicated in his book "Drosophilidae" (1935) it is known that D. simulans is absent among the palearctic forms of the Drosophila species. In the summer of the year 1936 we found D. simulans in Batoume (Adzharistan). A cytological analysis of salivary glands of the F_1 of flies caught in nature disclosed that we were dealing with hybrids between D. simulans and D. melanogaster. Further work yielded pure strain of D. simualans. Individuals from this strain were crossed with the American form of D. simulans. No difference between the two sets of salivary chromosomes could be detected.

Hadorn, Ernst. Pseudopupae. This term is suggested to be used for all cases where only a more or less normal puparium is formed within which no development of the imaginal discs occurs. Such pseudopupae are for instance formed by "lethal-giant" larvae and by hybrid males of D. melanogaster and D. simulans. Formation of pseudopupae in normals can be experimentally induced by injecting mature "ring-glands" into immature larvae. Pseudopupae may vary in their form. The best developed ones are like normal pupal cases, the poorest show only a hardening and darkening of the larval skin.

Hollander, W. F. Bithorax alleles. The mutants bx^W and bx^D were both discovered in one female. Nothing like bithorax had ever been observed in the stocks before. Other bithorax stocks were obtained from Cold Spring Harbor, namely those containing bx, bx^{34e}, and bx^d. The following phenotypes were obtained in the various hybrids:

bx/bx^{34e} = nearly wild type, slight development of metathorax.
bx/bx^d = wild type.
bx/bx^W = nearly wild type, but some overdevelopment of methathorax, often asymmetrical.
bx/bx^D = same as bx^D alone.
bx^{34e}/bx^d = wild type.
bx^{34e}/bx^W = blend
bx^{34e}/bx^D = rounded, flat, wing-like halteres, but not very large. Otherwise wild type.
bx^W/bx^d = wild type
bx^W/bx^D = oval, flat, winglike halteres, fairly large; little if any metathoacic development; flight not vigorous, but possible.
bx^d/bx^D = same phenotype as bx^d homozygous.

From the above results, I have concluded that these five factors are alleles, with no seriation of effect. No attempts have been made to analyze the salivary gland chromosomes.

Just, G. and F. Steiniger. Natural selection in D. melanogaster (normal-winged and vestigal) on the isle Greifswalder Oie. The investigations on selection under natural isle conditions (DIS-7, p.91) are continued on the isle Greifswalder Oie. They were also begun in the part Gellen of the isle Hiddensee.

Kaliss, Nathan. Determination of the color of malpighian tubules in larvae. Poulson has shown that the pigment of the malpighian tubules appears in zygotes that are 20 hours old. With a magnification of 440x, the color can be seen to be due to the presence of discrete yellow spherical particles located in the walls of the tubules. With a magnification of 1500x it was determined that the absence of color in genetically white first instar larvae is due to the absence of granules, either yellow or white, from the walls of the malpighian tubules. These observations were made on larvae dissected in Ringers' solution.

Kaliss, Nathan. The larval expression of the gene for yellow.

While observing male zygotes, 24 hours or older, that were deficient for the loci yellow and achaete, it was noticed that the mouth armature was yellow, as distinguished from the pale gray or black mouth parts of non-deficient wild-type eggs. Examination of genetically yellow late zygotes and first, second, and third instar larvae showed that the mouth armature was brown-yellow as contrasted with the black of non-yellow animals. The color darkens progressively with age.

The accuracy of this distinction was tested in the following manner:

1. From the cross y w/♀ x +♂, 40 first instar larvae were selected as phenotypically wild-type by their mouth parts. From these larvae 39 adults were recovered: 25 +♀♀, and 14 +♂♂. From the same cross 39 first instar siblings were selected as having yellow mouth parts. From these larvae 38 imagoes, all yellow white males, were recovered.

2. From the cross w/In-49, y Hw♀ x +♂ 19 second instar larvae were selected as yellow. These were recovered as 19 In-49, y Hw males.

3. A large number of 3rd instar larvae from the crosses (y ac)$^-$B/In-49, y Hw♀ x +♂, and w/In-49, y Hw♀ x w♂ were put on a slab of food. After they had worked through the food for half and hour, and presumably had become thoroughly mixed, 15 non-yellow and 15 yellow larvae were segregated. From the 15 non-yellow larvae, the following 15 imagoes were recovered: 4 wild-type ♀; 5 white ♀; 6 white ♂. From the 15 yellow larvae, 14 imagoes were recovered: 3 In-49, y Hw♀; 3 y Hw B♀; 8 In-49, y Hw♂.

It is interesting to note that Muller's classification of the mutation yellow as hypomorph is borne out by the appearance of the mouth armature in the hemizygous eggs deficient for the loci yellow and achaete. In these zygotes the armature is yellow. Miss Katherine B. Brehme has independently discovered the larval expression of the yellow locus while working on attached-X yellow larvae.

Komai, T. Collection of D. simulans from Japan.

In December 1936, D. simulans has been collected by Mr. K. Daido from Titizima and Hahazima of Ogasawara Islands (Long. 142° E.; Lat. 26-28° N.). This may be the first record of capture of this species from Asia.

Liebach, W. Gene manifestation.

Since 1936 the manifestations of a variable wing-gene (vli) shortening the longitudinal veins has been under examination. In the first place the different forms of the manifestation were divided into qualitatively distinguishable classes. Further investigations concern the influence of alcohol upon D. melanogaster.

Morgan, L. V. A compound duplication of the X-chromosome of D. melanogaster.

Crossing-over and disjunction have been studied in two lines of a duplication (Dp-100) in which the extra fragment is a deficient X-chromosome containing a distal section (X^d) from the y end to prune inclusive and a proximal section (X^p) from fu to the spindle fiber attachment. In one line (1,1) the fragment is attached to one X at spindle attachment and in the other line (1,f) the fragment is free on its own spindle attachment.

Crossing-over between the two entire X's was less frequent than in the diploid control, as in other duplications. In the region homologous to X^p the reduction in crossing-over is proportional to the length of X^p when compared with the proximal Dp-138 and other duplications studied by Dobzhansky (Studies III '34). The reduction in this region is the same in both lines of Dp-100. In the region homologous to X^d the reduction is very slight and is much less than in distal duplications (carrying some of the inert region) of comparable length. In the 3rd region, cv-ct (not homologous to the fragment), crossing-over is as frequent as in the control in the (1,1) line and is still more frequent in the (1,f) line. In the (1,1) line when a Y-chromosome is present, crossing-over is still more reduced especially in the most proximal region. Crossing-over of the proximal fragment (X^p) is only 0.3 times as frequent as crossing-over between the X's in the homologous region in line (1,f) and only about .08 times as frequent in line (1,1). Crossing-over within the distal fragment (X^d) rarely takes place.

Non-disjunction of X's occurs in about 3.5% of gametes in the (1,f) line. The X's of XXY females are usually non-crossovers, but a small percentage in one experiment were crossovers for a distal region. It is computed that non-disjunction of X's occurs in about 31% of no-exchange tetrads. Non-disjunction of X's in line (1,1) was infrequent being about the same as in XX controls. When a Y was present in the (1,1) line there was about 19% of non-disjunction which is 56% of estimated no-exchange tetrads.

Moriwaki, D. Drosophila repleta found in Tokyo.

In Tokyo, where D. repleta had never been found, the flies were first collected last year, 1936. Mr. S. Uchida, a student of Tokyo Imperial University, collected a few of them on November 13, 1936 at Shibuya-district in

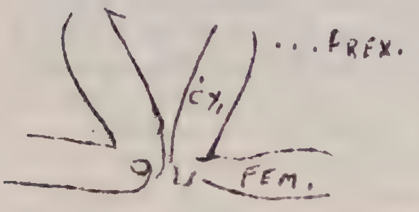
Tokyo, after that we caught them occasionally even in winter, for example on Dec. 29, 1936; and all of them were cultured by corn-meal-agar method. That they were certainly D. repleta was secured by mating them with D. repleta in America which was sent to me by Dr. Kikkawa in Kyoto. On Dec. 12, 1936, one female and six males of scarlet eyes appeared in my culture, and it was a mutant character caused by an autosomal recessive gene, which I named as scarlet. This summer, however, the stocks of wild and scarlet were at the point of death, but fortunately we could capture again the flies in nature in Tokyo on August 25, 1937. Then the culturing of wild and scarlet stocks of D. repleta obtained in Tokyo is now continued.

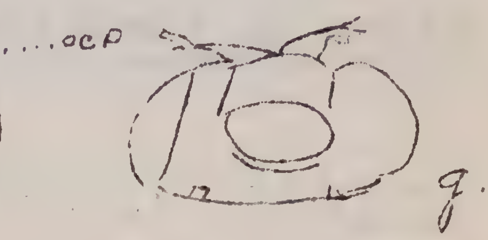
Neuhaus, M. Crossing-over in the bobbed region.

In order to study the frequency of crossing-over in the bobbed region the following crosses were undertaken. Females possessing y sc^4 (y sc^4- a long inversion, the right break occurring in the inert region to left of bobbed) and y^2 w^a f bb Y^L were mated to f Y^S Y^L males. F_1 y sc^4 males were tested for fertility. In general those males are sterile, but if an ordinary crossing-over to the right of the inversion takes place then a fertile y sc^4 male arises. And if the X-chromosome of that male contains bb then this indicates that the cross over has taken place to the right of the bobbed locus. 1500 y sc^4 males were tested for fertility and among them one fertile male was observed. A genetical investigation of the X-chromosome of this male showed that the X-chromosome contain bb and the long arm of the Y attached. Similar experiments were carried out with one sc^8 inversion and a testing over 5000 sc^8 males for fertility, but only sterile males were obtained.

Serebrovsky, A. S. On some newly appearing bristles.

A detailed study of s^9L-Ry^4 flies has revealed in the latter the appearance of some new bristles, designated by the author as praecoxales and occipitales. The former (prex) arise on the lower surface of the thorax anteriorly to the anterior coxae, one on each side, often asymmetrically and for the most part in homozygous females. Those bristles are fine and more slender and shorter than macrochaetes, being usually directed outwards. The latter (ocp) develop on the posterior surface of the head, one on each side, between the edge of the eye and the dark "trapeze". Those short bristles are directed upwards. Apart from the above said, there takes place a doubling of bristles, to which the term of genales (g) is given by the author. When examining the head from beneath, those bristles seem to be directed backwards. The occurrence of the above bristles is apparently caused by the doublication of loci ac^+ and sc in the chromosome of that structure. It is of interest, that flies having deletions, often display the appearance of the same bristles, probably due to a similar cause of hyperploidy for sc and ac. The study of several deletions has shown each of them to exert a characteristic influence either on prex or on ocp and g. Having compared eleven different deletions, the author was able to distinguish easily some of them according to those characters, when examing groups of flies. Such a circumstance may be utilized in working on deletions. Praecoxales are also to be observed in Hw and in h flies.





Serebrovsky, A. S. Interaction between the genes divers and yellow and scute.

V. V. Sakharov, by whom the divers mutation in D. melanogaster was found, detected the appearance of a new character in y div flies, viz. a strong upcurling of wings, more pronounced than in flies Cy. That phenomenon was studied by the author in combinations of div with other allelomorphs of yellow. In y^3 div (yellow body, black bristles) the wings get curved as strongly as in y^1 div. In y^3 div the wings are seen to curve somewhat less, but still very strongly. In y^N div (gray body, yellow bristles) (see Neuhaus DIS-4) the wings are either flat or slightly curved as in some y^+ div. Thus the degree of the wing curving is parallel to the body color ($y = y^3\ y^{3P}\ y^N$), showing no connection with the color of bristles. At the same time some allelomorphs of scute and achaete were investigated. The sc^3 div flies are of a very poor viability, the same being the cause of the failure in obtaining sc^{10} div. In sc^3 div, sc^6 div and sc^8 div the wings were found to be flat.

Shapiro, N. The frepuency of the somatic mosaic occurrence in males and females.

The writer has observed the frequency of the mosaic occurrence in the stock h ss/+ after X-raying heterozygous larvae. The latter were treated at the age from 3 to 48 hours from the moment of egg laying. The dose of irradiation was 1000r. The results are summarized in a table.

	Number of flies	Number of mosaics hairy	 spineless	Total
♀♀	1420	3	6	9
♂♂	1132	3	5	8

From the above given data it may be seen that the frequency of the mosaic occurrence for autosomal genes is alike both in males and females.

Surrarrer, T. C. Time of pupae case coloration.

In obtaining pupae cases of known age of a vermilion mottled eyed mutant strain at 27°C the pupae cases do not undergo any noticeable darkening during the first hour after pupation.

Waletzky, E. A haploid mosaic of D. melanogaster.

In a cross between W +/+ wp p^p x wp p^p ♂ a single female was found in which thorax, abdomen and the right side of the head were W +/+ wp p^p. The left side of the head was smaller than the right side. The left eye was peach in color and approximately two-thirds the size of the normal, red, right eye. The left posterior ocellus was peach and approximately half the size of the normal, right posterior ocellus. The arista and all macrochaetae and microchaetae on the left side of the head, were not more than half the size of those present on the right side of the head.

Whittinghill, M. Salivary studies on translocation.

Salivary analyses (and supplementary tests as to the localizations of several mutants on the salivary map) have been obtained in a series of Y-2 translocations (found and first used by Dobzhansky: Biol. Zbl. 50:671-685, 1930; also Z.i.A.V. 60:235-286, 1932) and in T(2,3)Moiré (formerly Me^{1x}).

Three of the translocations, T(Y;2)A, B and C, were indistinguishable from each other in the salivaries, though differentiated genetically by crossing-over relations with thick (Dobzhansky) and by a position effect with rolled (see below). In each of these three translocations the break in the second chromosome was found to be just before band 41A1 of Bridges' 1935 map. Synapsis in 2R was greatly interfered with, especially near the region of the break.

Translocation D was found to be a complex rearrangement involving the third chromosome also and is, therefore, here designated as T(Y;2;3)D. Six(or more) breaks were found in the second and third chromosomes as follows: before 30A1, before heaviest band of 34C, undetermined breaks (or break) proximal to 41D, before 61F1, before or after 62A5 (which is the last of four similar light bands), and in 78F. The new arrangement of chromosomes was found to be as follows: Proximal part of Y; 29F to 2L tip. - Distal part of Y; 30A1 to 34C; 78F through spindle attachment to 3R tip. - 3L tip to 61E2; deficiency of 61F1 to 62A4 or 5; 62A5 or 6 to 78F; 34C to spindle attachment; unanalysed rearrangement in 41A to C; 41D1 to 2R tip.

Translocation (Y;2) E was found to have its break between the two heavy capsules of 36D. This is to the right of the locus of black, rather than to the left as determined genetically by Dobzhansky.

Translocation (Y;2) I was found to contain exchanges between 3 and Y as well as between 2 and Y, so it was thereupon designated T(Y;2;3)I. The salivaries showed a break in 2R after 47A1 capsule and five breaks in the third chromosome, after 69C2, between 74A1 and B1 capsules, after 84E1 or 2, between the heavy doublet of 91E and in 99C. The new order of segments was found to be: 2R tip to 47A; s.a. Y; 91E to 84E; 69C to 74A; 99C to 3R tip. 2L normal through s.a. to 47A1; tip of one arm of Y. Tip of other arm of Y (presumable, not seen); 91E to 99C; 74B through s.a. to 84E; 69C to 3L tip.

Translocation (Y;2) J as studied by both Bridges and this writer revealed an inversion in addition to the translocation. The entire 2R is carried by the Y in this order: Y s.a.; 57F1 to 41A1; 57F2 to 2R tip.

The Moire used as a balancer for the entire third chromosome, formerly called Moiré^{llx} (DIS-3:13 Pub. Glass '33. J. Gen.28:104) was found to require the designation T (2;3)Mé. It is composed of the translocation of the tip of 2R to the spindle attachment of 3, the inversion, of an adjacent segment of 2R, the Payne inversion (carrying Mé), a new In(3R), plus a reciprocal exchange between L and R arms, i.e., a central inversion extending across the s.a., which cuts into the L and R inversions. Breaks were found at eight places, as follows: through 48C1 capsule, after 59D1 capsule, after 63B1, in 69E, in 72E, in 89B, in 91C and in 97D. The altered chromosomes were found to have the constitution outlined below:

2L normal through s.a. to 48C1; 59D1 to 48C2. 3L tip to 63B1; 72E to 69E; 91C to 97D; 89B through s.a., which bears 59D to 2R tip seemingly as a lateral attachment; along base of 3L to 72F; 63B2 to 69E; 91C to 89B; 97D to 3R tip.

Genetic studies were undertaken on some of these, chiefly T(Y;2;3)D, to determine the nature of phenomena not explained on cytological grounds, such as the difference between Translocations (Y;2) A, B, C and J, and the pale and the Minute characteristics of the T (Y;2;3) D phenotype. The results of matings of translocations to genes located at the base of 2R can best be presented in tabular form.

Translocations: genes	A	B	C	D	J
lightoid	x	x	x	x	x
blot	x	x	x	x	x
straw	x	x	x	p.e.	x
rolled	x	p.e.	x	x	x
thick	x	x	x	x	x
apterous	x	x	x	x	x
Minute-p	-	-	-	allel	-
misformed	x	x	x	x	x

Legend: p.e., position effect found; x, no effect; -, not tested satisfactorily.

The position effect of T(Y;2)B with rolled was found by Mrs. V. Curry, others by the writer.

Breakages in other regions of the second chromosome showed no position effects in the following series of matings: T(Y;2)J with wt, sm, hy, a, px; T(Y;2)E with j, lm, el, rd, pu, an, cru, rh, ck, hk, bri; T(Y;2;3)I with en, upw, chl; T(Y;2;3)D with Mz, Sk, cl, pi, Sp, spd, gt^4, tkv, d, tkd, J, ab.

After the cytological discovery of a deficiency around the locus of roughoid in T(Y;2;3)D, matings were made with all mutants in this vicinity to find out whether the deficient material might not be present somewhere else in the nucleus and, if not, what other loci it might include. It was found that this is a true deficiency and that the loci of anarista, rougnoid and veinlet (but not Roughened) are included within its limits, 61F1 to 62A4 or 5.

Zimmer, K.G. and N.W. Timofeeff-Ressovsky. Production of mutations by neutrons in D. melanogaster.

A statistically significant increase of the rate of sex-linked mutations in D. melanogaster (ClB-method) was obtained by irradiation with neutrons (0, 96% ± 0, 20, as compared with 0, 19% ± 0, 07 in the controls) from an "artificial source (Philips, Eindhoven). Against all other radiations (X-rays produced by the neutron-apparatus) the flies were protected, so that the whole difference in the mutation rates (0, 77% ± 0, 24) is due to protons secondarily induced within the flies by neutrons. Dosage-work (determination of neutron-irradiation-dosages in r-units, equivalent to those of X-rays), as well as further irradiation-experiments are in progress, and will allow an exact comparison of the effectivity of equivalent dosages of neutrons and X-rays. The last question is of interest in connection with the problem of the influence upon the effectiveness of the total dosage of the time-and space-distribution of ionization along the path of the secondary electron or particle.

Technical Notes

Bridges, C. B. Concentration of moldex in culture media.

A concentration of 1.0% of a 10% alcoholic solution of moldex (Moldex-A from Glycol Products Co., 148 Lafayette St., New York, N. Y.) was used at Pasadena for some months in culture media (DIS-6:62) for several species of Drosophila. It was found to control mold perfectly, but was reported by several workers to give fewer fertile cultures, lowered productivity and smaller flies - presumably through hindering growth of live yeast. For the past year a concentration of 0.7% of the solution (0.07% of the chemical) has been used. This is adequate to keep molds from appearing and seems not to give bad effects on the flies.

Bridges, C. B. On the seeding of culture with yeast.

On some five occasions the method of seeding the surfaces of culture bottles by spraying with very thin yeast suspension (see DIS-6;66) has been hopefully put into practice and then abandoned in favor of seeding with one, or better two fat drops of thick yeast suspension. Always the failures of pair cultures to produce offspring became so large as to seriously hinder the experimental breeding. The main advantage of the spraying was the suppression of mold growth - and this is now better accomplished by moldex.

Bridges, C. B. On distinguishing larvae for salivary preparations.

The notes by Beadle (Am. Nat.71: 277; DIS-6:24), Hoover (DIS-6:24) and Brehme DIS-8: -) show how the distinction between the yellow of normal malphigian tubules and the colorless or paler tubules associated with certain light eye-colors (notably w and lt but also cm, g^2 p^p and ca) and the brown color of the mouth parts of yellow larvae, can be used to select larvae of the type desired for salivary preparations. A survey of our balancers shows that a few of them are especially useful for general use in balancing any mutant whose salivaries might need investigation. For chromosome I, these are: Cl, y Hw (carrying y and g^2) dl-49, m^2 g^4 (carrying g^4, already recommended by Hoover) and dl-49, w lz^s. For chromosome II, the best is Cy, al^2 lt^3 L^4 sp^2 (carrying lt^3 of Beadle) and for chromosome III, the best is Payne, Dfd ca. These balancers are the best of the ClB, dl-49, Cy and Payne varieties, and should be kept on hand and favored in stock making.

For second and third chromosome aberrations, use can be made of the dominant eye-color p^G which has pale tubules (perhaps p^G/p^p are better). First cross to T(Y;2;3)p^G, pick out the T(2;3)p^G aberration ♂♂ and cross to p^p ♀♀ , using the normal yellow tubuled larvae.

A second and third chromosome method needing no distinguishing of larvae, except the easy one of femaleness, has been the use (by Schultz and myself) of T(Y;2;3)I. Cross the female bearing the aberration to Ø males carrying T(Y;2;3)I, pick out sons carrying the aberration (all are T(Y;2;3)I and cross to any standard female. All daughters are heterozygotes for the aberration.

Buzzati-Traverso, A. Method for making salivary gland chromosomes permanent smearing.

I found very convenient for making permanent salivary gland chromosome smears the following method: (1) Dissect as usual the larva and leave the salivary gland in normal aceto carmine till well stained; (2) pass the gland to the slide and take off all the aceto carmine which might be on the slide, avoiding the gland to dry; (3) let two or three drops of the following liquid fall on the gland; 1 part Faure's liquid (50cc. of distilled water + 50cc. of chloral hydrate + 20cc. of glycerine + 30cc. of arabic gum) mixed with 2 parts of normal aceto carmine (the proportion 1:2 should be changed slightly to fit the different materials); (4) put the cover slip on and smear as usual, leaving the liquid in excess to dry around the edges of the cover slip. The slide will be dry enough to be used in a few hours, and keeps in very good conditions practically indefinetely.

Just, G. and F. Steiniger. Food.

The marketable fruit-marmalades, which could be used as a convenient Drosophila food, in Germany nowadays contain remedies for preventing fermentation, and the food becomes very acid in short time. These marmalades are to be used only with great cautiousness.

Lüers, H. The use of the dominant Bobbed in the Y-chromosome of D. funebris in genetic experiments.

A dominant bobbed-mutation was induced by X-rays in the Y-chromosome of Drosophila funebris (Bb^y). Some properties of this new mutation make it useful in some genetic experiments. (1) Bb^y has a markedly slower development. Since it is present in males only, it facilitates the obtaining of virgin females from mass-cultures. (2) Bb^y is a good marker of the Y-chromosome. (3) Bb^y enhances non-disjunction of the X-and Y-chromosomes, and facilitates the obtaining of XO- and XYY- ♂♂ and of XXX-, XXY- and XXYY- ♀♀.

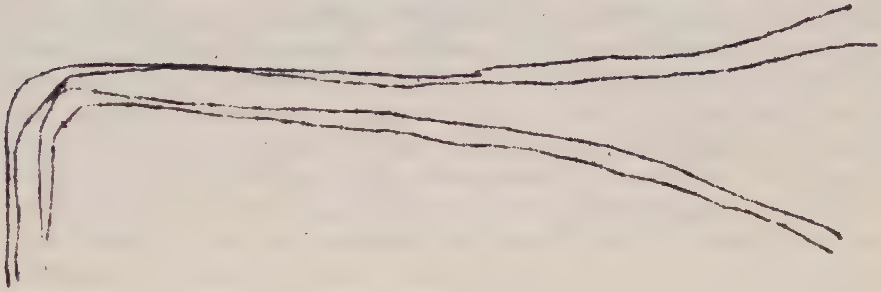
Medvedev, N.N. How to make Drosophila larvae immobile for a short time.

In order to carry out transplantation experiments on Drosophila larvae it is necessary to make them for some time immobile. Beadle and Ephrussi in their work use the current method of etherization. This treatment, however, is very undesirable, because it makes transplantation experiments tedious by themselves still more difficult, especially in the case when they are carried out by but one person.

For this purpose the author successfully uses a very simple method. After placing a larvae on the slide where we are going to perform the transplantation, it is quite sufficient to press it gently with a piece of filter paper and to roll it over a few times around its longitudinal axis. After this simple manipulation the larva becomes immobile for a time sufficiently long to perform a transplantation.

Medvedev, N.N. Pipette in Drosophila transplantation experiments. In distinction from the pipette described for transplantation experiments on Drosophila by Beadle and Ephrussi we are using the pipette represented in our text-figure.

The very end of such a pipette during performing transplantation is wholly visible in the field of a microscope in a horizontal plane. This peculiarity provides the possibility of checking the movement of an organ under implantation within the capillary of the pipette and at the same time to check more precisely the quantity of physiological solution injected.



Spencer, W. P. Factors involved in oviposition. As an increasing number of workers are undertaking problems involving the collection and hatching of Drosophila eggs (transplantation experiments, study of larval lethals etc.) a few notes on factors leading up to and inducing oviposition may prove of interest.

(A) Pre-feeding of females. Starved flies will lay few or no eggs. It is important to furnish flies which are to used in egg laying experiments an adequate supply of fresh food, particularly on the day or two days prior to the collection period. It is also well to use flies which have been matured for several days to two weeks depending on the species. If flies are aged in vials fresh food chips should be added or the old ones so cut as to furnish fresh surface, as the surface of a food chip in a vial soon dries or forms a film which cuts down on food consumption.

(B) Humidity. To elicit the ovipositing reaction the air in contact with the surface where the eggs are to be laid must have a high humidity, probably close to or at the saturation point. This condition is frequently supplied, but sometimes unwittingly. Enclosing moist food medium in a glass container tends to supply the proper humidity. However, a small paper spoon of medium in a half pint bottle, particularly in a dry climate, may not raise the humidity sufficiently to secure optimum results. The scraping and roughening of the food surface described by a number of workers supplies tiny humid valleys where the ovipositor meets an environment sufficiently moist to induce the reaction. However, eggs will be deposited in or on a smooth surface if the air in contact with it is saturated with moisture. Conversely no eggs are deposited in dry air. In a dry climate fresh medium may be left exposed to flies which will feed on it but will no oviposit. If, however, the same dish of medium is covered so that the humidity rises oviposition occurs. Thus in nature flies invariably oviposit in cracks or on the under surface of exposed food.

(C) Temperature. Ovipositing occurs through a wide range of temperatures differing somewhat for different species of Drosophila. The range however, is not as great as the upper and lower limits of temperature to which the fly is tolerant. Thus females which have been well-fed may be kept at temperatures below 10C for long periods of time without ovipositing. When the temperature is raised the first eggs laid are small and abortive, indicating a resorption of material from eggs held in the uterus for a long period. Roughly the temperature range is somewhere between 10C and 30C with the optimum differing for different species.

(D) Medium for oviposition. When all other conditions are satisfied, i.e. females properly aged and well-fed, temperature neither too high nor too low, and humidity conditions correct, flies will oviposit readily on a great variety of substances from the most elaborately prepared media heavily yeasted to cellucotton or tissue paper soaked in distilled water or tap water. The writer has collected eggs on cellucotton soaked in sugar-water, or yeast solution, or water alone, on raw beet, raw potato, various fruits such as apple, pear, banana, moist oatmeal, moist bran and the usual media. Stangely enough, all previous notes on the collecting of eggs including notes of the writer in DIS-7 mention yeasting the surface as a necessary part of the routine. Actually yeasting has little or nothing to do with inducing oviposition. If the same surface is made to do triple duty as food for the parent fly, ovipositing medium and food for the larva hatched from the egg, then possibly yeasting is indicated. However, the logical time to do the yeasting would be after the eggs have hatched or are about to hatch, as overgrowth of yeast is likely to cover and smother the developing egg. This is particularly true of small, slow-hatching eggs, deeply buried in medium. It is quite possible to provide in the same vial one surface for the feeding of the flies and another of quite a different nature for the collection of a large part of the eggs laid. Where the same surface is to be used for the three purposes mentioned above, and young larvae rather than eggs are desired the writer recommends small wads of cellucotton soaked in sugar water. This serves as an ideal feeding surface for adult flies, and reduces to a minimum the danger of weak adults sticking and drowning. The moist, porous surface induces oviposition. The addition of fresh baker's yeast dissolved in water or for some species in a Ringer's solution containing particularly Mg and K ions brings the larvae through to pupation under optimum feeding conditions. The dissolved yeast may be added to the cellucotton at about the time the larvae hatch. Eggs or larvae may be secured for study or experiment at any time by shaking a bit of the cellucotton in Ringer's solution.

Spencer, W. P. The use of cellucotton in Drosophila culture.

A porous cellulose compound under the trade name, Cellucotton, furnishes and excellent base for culture media for Drosophila. This material comes in large bats, 2lb, 5lb, and 8lb. It is extremely porous and absorbent. One gram will soak up and hold without dripping 20 cc. of water or other liquid media. The material may be readily cut into wads of convenient size and placed in any design of culture bottle. Liquid media of which the main constituent is a sugar, (cane sugar, either refined or brown, or molasses), together with salts such as are added in the culturing of yeast, may be poured or pipetted onto the cellucotton and the surface seeded with a little powdered yeast. Flies are then put in. If there is any trouble with molds, moldex or other mold preventatives may be used. At any time during the life of the culture food may be added either in the form of the original solution or of baker's yeast in liquid suspension. It is also possible to raise the larvae from the start on yeast suspension, to which for some species salts must be added. In this case a small wad of cellucotton soaked in sugar water should be stuck to the side of the culture vessel as food for the parent flies.

If larvae are raised on yeast alone be sure to add a wad of cellucotton soaked in sugar water before emergence of adults as they will not live long on a yeast diet.

The advantages of the cellucotton will be obvious to anyone using it. There is no cooking of food media necessary. There is no necessity for cutting out a food plug to allow escape of CO_2. There is less tendency for flies to become stuck in the food medium. There is a more effective use of the media by the larvae and an increased yield per culture bottle. Large, well-nourished larvae are more readily available for salivary chromosome study. Much smaller containers can be used for rearing a given number of flies, thus cutting down on incubator space necessary for running an experiment. With proper handling of adults as to numbers and time left in culture overcrowding should not occur. When this is allowed to take place more cellucotton soaked in yeast may be added.

Steinberg, A. Micro-burner. A cheap and easily prepared micro-burner can be made by cutting away the sharpened end of a hypodermic needle and mounting the remainder on a block of wood. A burner prepared from a B-D gauge 27 needle has been found to give excellent results in the preparation of needles for the Ephrussi and Beadle transplantation technique.

Stern, Curt. Methylene blue - staining of peripheral nervous system in D. melanogaster. Every macro- and microchaeta of Drosophila is innervated by a peripheral nerve cell. The centripetal axons of these cells join into nerves which form a characteristic pattern underlying that of the setae. Method: Very lightly etherize adult flies. Inject into the sternum, by means of a fine pipette, a solution of methylene blue BX-Gruebler (1% in 0.7% saline). Enough fluid should be injected to make the whole animal appear blue. After 20 minutes to 2 hours dissect desired parts, such as whole head or dorsal half of thorax, in cold 10% ammonium molybdate solution. Leave in this fixative fluid for 2 or more hours. Make total mount according to ordinary technique. - This is not an original method except for its application to Drosophila. It is not invariably successful.

Tanaka, Y. and T. Takami. New food material. We tested Koji (malted rice), rice-bran, wheat powder, banana, dried fruits, etc., and found that rice-bran might be the cheapest material in Japan giving good results. An example of our rice-bran food formulas is as follows:

Water	1000 cc
Rice-bran	80 g
Brown sugar	60 g
Agar-agar	20 g

Heat agar in water until it dissolves, add rice-bran and sugar, then boil the mixture during some ten minutes stirring well.

News

Seventh International Congress of Genetics. - "The question as to the place for the next International Congress of Genetics has now been considered and voted upon by the International Committee of Genetics Congresses. The International Committee has by a large majority resolved to invite the British geneticists to arrange the next congress in Great Britain in 1939. The committee of the British Genetical Society has passed a resolution welcoming the invitation. The British Geneticists will take the earliest opportunity of appointing an Organization Committee which will send out further information concerning the date and place of the congress." - (O. Mohr).

Reprints of Drosophila genetics articles wanted: Having just started Drosophila research work for the first time in Italy, I shall feel very grateful to any research worker who will send his reprints in exchange of the articles which shall be sent from my laboratory. Also the old reprints will be welcome. - (A. Buzzati-Traverso).

Due to prolonged delays in completing spraying apparatus for testing contact poisons, our experiments using Drosophila [illegible] vestigial flies for the biological assay of the toxicity of plant extracts of insecticidal value are just starting. Experiments with local food media have been completed and are being prepared for publication. They were unusual in applying Yates' method of randomized pairs for eliminating differences in productivity between individual females from the estimate of error and have led to a simplification and improvement of the medium used elsewhere in Leningrad. (C. I. Bliss).

Additional Reasearch Notes

Pollitzer, Otto. Crossingovervariabilität.

Bei Untersuchungen über Crossovervariabilität an einem III ple Stamm mittels Rückkreuzung nach dem Schema se gl^3/cp e^{11} x se cp gl^3 e^{11} zeigte sich bei einer Versuchstemperatur von 25 Grad C. und einem Weibchenalter von uber 36 Tagen ein Absinken der Austauschwerte. Angaben über ein Verhalten in dieser Altersstuffe habe ich bisher in der Literatur nicht gefunden. Die Altersvariabilität zeigte maximale Abweichungswerte in der Region se-cp minimale in der gl^3 e^{11}. Weitgehende Unterschiede im Crossoververhalten fanden sich bei extremen Zuchttemperaturen. Die Interferenz zeigte ein ständiges Ansteigennmit dem alter bei 31 und 25 Grad C. Zuchttemperatur, bei 15 Grad C. hingegen ein anfängliches Absinken der Werte gleichzeitig mit einer Verringerung der Internodienlange, nach dem 20. Lebenstag machte sich aber auch hier ein Ansteigen der Interferenz mit dem Alter bemerkbar. Neuere Berechnungen der Lokalisation der Mutation cp III chromosome (fruher ci Mainx) ergaben einen Wert van 46.5 ± 0.8.

Seventh International Congress of Genetics - In accordance with a resolution of the International Committee and with the decision of the Organizing Committee elected by the Genetical Society of Great Britain, the VIIth INTERNATIONAL CONGRESS OF GENETICS will meet in Edinburgh in 1939, probably from August 23rd-30th inclusive. Professor F.A.E. Crew, Institute of Animal Genetics, University of Edinburgh, Edinburgh 9, has been appointed General Secretary to the Congress and to him all correspondence concerning it should be addressed.

Research Notes

Argelander, A. and E. H. Strasburger. A case of genetic disfunction of the ovaries.

In a cross between ♀ ClB sc^{1} v/y^{2} sc^{8} w^{a} X ♂ y^{2} sc^{8} w^{a} males were found with eyes considerably lighter than w^{a}, similar to w^{a}v. Seven of these males were mated to y^{2} sc^{8} w^{a} ♀♀ from the same culture. Three matings were without light-eyed flies, whereas in 4, half of the flies were y^{2} sc^{8} w^{a} and the other half had light eyes. The inversion made it impossible to analyze new eye color. Crossing with y^{2} sc^{8} w^{a} v showed, however, that v was not present in the new mutant. The light-eyed females proved to be sterile. 56 homozygous light-eyed females mated to light-eyed or Berlin wild stock males gave no offspring. y^{2} sc^{8} w^{a} ♀♀ heterozygous for the light eye color gave as many flies with w^{a} as with light eyes. The light-eyed males proved to be fertile. The stock is since kept by crossing light-eyed males with females heterozygous for the light color. The histological investigation of homozygous and heterozygous light-eyed females was done by E. H. Strasburger. The ovaries, together with their adnexes, were fixed in Carnoy and stained according to Feulgen method. Preparations were made of virgin light-eyed females and of females which had been mated to light-eyed males. In both cultures no eggs were found. Result of the microscopic investigation: The ovaries of all the homozygous light-eyed females are obviously abnormal. However, they are normal as far as the number of egg tubes per ovary, the number of sections per tube and, though perhaps not always, the number of cells per section. The nuclei of the older egg compartments are always, those of the younger ones often absolutely pycnotic. In the older cells there is obviously plasma constriction. In the egg ducts often pathological heaps of eggs and nurse cells are found. The three spermathecae and the two paravaria are normal and always present. The heterozygous females were found to be normal.

Buzzati-Traverso, A. A sex-linked modifier of brown.

In the F$_{2}$ of a cross between a y ♀ and a bw; e wo ro o, flies appeared with rose eyes. This eye color proved to be due to a sex-linked recessive modifier of brown, which, when homozygous in presence of homozygous bw produces the rose eye color, and when homozygous in presence of heterozygous bw lowers the dominance of the normal allel of bw so that the flies carrying such gene combination are brown. Experiments are in progress to find out whether it is a specific modifier and to localize it in the X-chromosome.

Buzzati-Traverso, A. Direct proportionality between X-ray dosage and translocations between the 2 and 3 chromosomes of D. melanogaster.

Using the L^{2}/Cy - 3-ple method to detect translocations between the second and third chromosome and X-ray dosage of 1500, 3000 and 6000 r (the irradiation being made in Berlin by K. G. Zimmer) it has been found a direct proportionality to the quantity of applied irradiation. 3,59±0,75, 6,14±1,82 and 12,21±1,83 are the percentages of translocations obtained with the three mentioned doses. Experiments are in progress to obtain more points along the curve, to check the wave-length and the time-factor effects.

Brasted, Adair Multiple effect of "engrailed" in D. melanogaster. The mutation "engrailed" in D. melanogaster was described by Eker in 1929 (Hereditas 12:217). He enumerated two of its effects: (1) a longitudinal split in the scutellum extending anteriorly from the posterior border, and (2) shorter, broader and more thin-textured wings than in wild-type flies, with numerous abnormalities in venation. A third, hitherto unreported effect, discovered in October 1937 by Dr. Curt Stern, is the production of an extra sex-comb on the lateral side of the first tarsal joint, directly opposite to the normally present sex-comb. This extra sex-comb has fewer, more irregularly arranged teeth than the normal, and is considerably more sensitive to environmental variations. Its size has been found to vary inversely with temperature. The "split scutellum" was reported in DIS-7:20 as a new mutant (spc), due to erroneous labilling of a stock. It is now obvious, however, that this condition is one of the multiple effects of "engrailed".

Crew, F.A.E. and Rowena Lamy Mosaics. Mosaics of D. pseudo-obscura carrying autosomal as well as X-chromosome markers were obtained, and it was found that the autosomal characters as well as the sex-linked ones were expressed in the exceptional regions, indicating that these were haploid for the whole chromosome complement. As the other mosaics obtained from the Plexus strain (v. DIS-8) were also probably haplo-diploid, their normality as to sex is explained, since haploid tissue has been shown to be female (Bridges 1925). Three male mosaics obtained in the same strain are clear cases of double-nuclear fertilization.

Gershenson, S. Investigation of genetical processes in wild populations of Drosophila. An extensive work along this line has begun during the summer and autumn of 1937 in a locality near Kiev (USSR). This work involves not only a complete genetical and cytological analysis of wild populations of two species (melanogaster and busckii) but also experiments on natural selection, rate of reproduction in nature etc., and an analysis of a number of ecological questions connected with the main subject. The work is to be carried on during several years.

Gershenson, S. Drosophila species near Kiev. The following species of Drosophila have been collected by the writer in Kiev and its suburbs during August-October 1937: (1) melanogaster (fasciata) (2) obscura (not pseudo-obscura) (3) busckii (4) phalerata (5) funebris (6) Spinulophila (Drosophila) immigrans (7) Parascaptomyza disticha (8) maculata (new species, name provisional). The first five species are numerous; 6 and 7 are rare; D. maculata is very rare. Most individuals of D. melanogaster and all of Parascaptomyza caught in nature were infected with the nematode Chondronema (family Anguillulidae, sub-family Sphaerulariinae).

Glancy, E. A. and R. B. Howland. Histology of developing bristles. A study of the histology of bristle growth in normal and mutant flies, especially in the mutants used previously for transplantation studies, is being carried on. The tormogen and trichogen cells in the mutant singed do not differ from wild type cells in relationship to each other or to the hypoderm, nor can they be distinguished from the wild type cells cytologically. Yet the singed bristle, from the moment it can be recognized (approximately 30 hours after puparium formation at 25° C.), is conspicuously stouter than the wild type, and is curved or otherwise distorted.

Goldschmidt, Richard. A note on so called mass mutation. Thus far 5 cases of so called mass mutation in Drosophila are on record (Spencer has in addition pointed to the existence of this phenomenon in a general way). Two of these have occurred in pure Florida stock (Goldschmidt '29, erroneously attributed to the simultaneous heat treatment, Demerec '37 ("explained" by a gene for mutation). The third (Plough and Holthausen '37) occurred in a Florida-cross. A fourth set of cases was found in Goldschmidt's plexus-blistered stock and a fifth by the same author in a cross bs x Oregon. In studying a certain position effect which is common to most third chromosome inversions Mr. Gardner found that crosses involving pure Florida stock produced the same effect. A salivary analysis by Mr. Kodani revealed a large inversion in the third chromosome of this stock (Meanwhile also found by others). The plexus-blistered stock (this is a purely descriptive name) is a very complicated translocation stock, as will be described in detail later. The standard bs, supposed to be an ordinary recessive, turned out, both genetically and in salivary analysis (latter by Mr. Kodani) to contain a translocation with strange position effects upon the bs expression. Thus all stocks which thus far produced the mass mutation phenomenon contained major chromatin rearrangements. A detailed description of our material is being prepared.

Green, Melvin. Variations in the expression of vesiculated-29c. The mutant vs^{29c} usually manifests itself as a liquid-filled vesicle in the region of the first and second posterior cells of the wings. Deflation of the vesicle soon after emergence results in a glassy, ruffled condition of the wing. Individual variation is frequently encountered in size of vesiculation; and in a few cases flies appear with one wing completely wild type. The effects of temperature on vs^{29c} are now being studied. Preliminary experiments in which development took place at a temperature of 30 degrees ± 1 degree C (except for a 2 hour egg-laying period at room temperature) gave the following results: 25/545 males or 4.6±1.03%SD had wild type wings; 53/452 females or 11.7±4.1%SD had wild type wings. The wild type males and females showed by tests to be genotypically vs. In two years handling of the stock at room temperature no males or females with both wings wild type have been observed.

Honer, E. Cytogenetic investigations on a complex dumpy. A single dumpy ♂ arose in the F_2 of a cross of X_8 x Oregon. This dumpy was found to manifest itself only within a special genotypical milieu where it behaves like a dominant, homozygous lethal. Flies which contain the milieu only have quite normal wings but tend to give phenocopies of dumpy when bred at higher temperatures (28-30° C). The milieu is built up by modifiers which are about to be classified. As dumpy gives a strong compound effect with the known dumpy (dp-2-13,0) (the wings are much more shortened and the legs knobby) it is supposed to be connected with a small deficiency at the dp - locus. Slides of salivary glands did not show any typical aberration near the free end of 2L.

Law, L. W. Radioactive phosphorous and the lethal mutation rate in Drosophila.

The results obtained by use of X-rays in influencing the structure of chromosomes in Drosophila have proved of invaluable aid to geneticists. It was thought worthwhile to attempt to influence the lethal mutation rate by radioactive phosphorous. A 1% solution of radioactive Na_2HPO_4 was obtained from Dr. John Lawrence of the University of California. This substance had been removed from the cyclotrone 15 days previously, so that at the time it was used it had a strength of 30 micro-curies per cubic mm. It gave off chiefly beta and gamma rays. A series of concentrations were then injected into 4 day old larvae of the Oregon-R strain (method of Beadle and Ephrussi) in order to determine the sub-lethal dosage. This was found to be 0.1%. Approximately 1 cubic mm. was then injected into Oregon-R males and lethals tested for in the usual ClB manner, using the stock of X-ple/ClB flies. No lethals were found in 250 tested X-chromosomes as compared with no lethals in 507 control chromosomes.

Ludwig, W. A lamarckian experiment on Drosophila

In the Zoological Institute is bred since June 1933, a stock of D. melanogaster Oregon +, called "Lamarck" (130 generations up to date). Immediately after hatching the flies have their wings and halters cut off. The purpose is to find out if the wing-muscles show a reduction on account of the wings not being used. According to investigations on double-hemithorax-flies (DIS-7:17) which, in spite of lack of whole thorax-muscular-tissues, are able to run and spring nearly in the same way as the wild ones, it is certain that the wing-muxcles are used for flying. Furthermore, from other species of flies which are not able to fly, it is known that this circumstance is accompanied by a reduction of the wing-muscles. An examination of "Lamarck" after 100 generations shows the following result in comparison to Oregon +, There is no difference in the total number of the nuclei in the muscles (= number of muscle-cells) the total number of fibres, and the total volume of the muscles, that is to say, up to now no influence in respect to lamarchism could be detected. The investigations are continued, both from morphological and physiological point of view.

Ma, S. Y. Temperature experimente an Drosophila melanogaster, insbesondere zur Bestimmung der sensiblen Perioden für die Induktion einiger Arten von Modifikationen.

Eier, Larven, Vorpuppen und Puppen eines lang ingezüchteten Oregon-Wildstammes wurden in verschiedenen Entwicklungsstadien mit Letaltemperaturen von 38,5° bis 41° C. gereizt. Es wurde dafür gesorgt, dass die Knostanz der gebrauchten Methoden (mindestens innerhalb eines Stadiums oder einer Versuchsserie), die Genauigkeit der Altersbestimmung und die Exaktheit der Hitzebehandlung stets aufrecht erhalten wurden. Die Experimente und die Auswertung des Ergebnisses sind noch im Gange, die bisherigen Resultate zeigen aber bereits folgendes: 1. Es besteht ein Geschlechtsunterschied in der Entwicklungsgeschwindigkeit, d. h., ♀ entwickelt sich schneller als ♂. 2. Die Hitzesterblichkeit ist hoch in früheren, niedriger in späteren Entwicklungsstadien; innerhalb eines Stadiums ist sie am Anfang und am Ende (einschliesslich der Häutungsperiode) gross, in der Zwischenzeit aber gering. 3. Es wurden in hohen Prozentsatzen verschiedene Arten von Modifikationen erhalten, welche spezifisch sind fur die Entwicklungsstadien, auf denen die Hitzebehandlung stattfand, und in einigen Fallen auch fur die angewandten Temperaturen und Reizzeiten. 4. Der quantitative Unterschied in der Sterblichkeit sowie in der Auspragung der Modifikationen zwischen den beiden Geschlechtern scheint auf dem Geschlechtsunterschied in der Entwicklungsgeschwindigkeit zu beruhen. 5. Die sensiblen Perioden einiger erielter Modifikationen (Die Zahlen genen die Anzahl der Stunden nach dem Eintritt der bezeichneten Stadien an; die unterstrichenen die optimalen Zeitpunkte. E = Embryonales Stadium; LI, II, III = 1.2.bezw.3.Larvenstadium; P = Puppariumbildung): Flugel: (1) Uberzahlige Querader, uberzahlige Ader und Queraderunterbrechung. Spates LIII-P 30. (2) Ausfallen von Randharchen. P 10-15-20. (3) Schmalflugelig (selten und asymmetrisch). P 15. (4) Dachformig (nur nach langer Reizdauer). P 10-30. (5) Abgestutzt. P 10-20-25. (6) Abwarts gekrummt. P 0-30-45. (7) Aufwarts gekrummt. P 35-70-85. (8) Gespreizt (stark wenn lang gereizt). Fast im ganzen Vorpuppen- und Puppen-Stadium. (9) Aufgerichtet. P 70-94. (10) Blasenbildung (lang u. stark gereizt). P 30. Augen: (11) Gefurchte Augenoberflache. Spates LIII-P 15. (12) Unregelmassige Verteilung von Facettenharchen (rauh). Spates LIII-P 60. Beine: (13) Verkruppelt. E 10-20; LIII 22-P 12. Borsten: (14) Verkleinert, gegabelt und gedreht. LIII-P 30. (15) Verzweigt. P 25-40. (16) Hakenformig. P 35-50. (17) An der Spitze geknickt, geknopft. P 45-55. (18) Schwach bis ganz entfarbt. P 55-80. Aristae: (19) Harchen querlig verzweigt. P 30-35-40. Thorax: (20) Eingedellt. P 70-94. Scutellum: (21) Hornartige Auswuchse. P 10-20-30. Innere Gewebe: (22) Tumorartige, schwarze Korperchen. LIII.

Muller, William A. Possible sex influence upon the expression of a dominant blister wing.

The mutant blister-like is a dominant located 3.2 units from bw (965 total count). Small counts indicate no extreme chromosomal fragmentation. The character mainly involves wing veination and excess liquid causing a blister. All individuals do not show a definite blister. Blistery may show a minute thickening of the veins, an absence of veins, or a definite blister of the wing. Usually only one wing has a blister; however, when one wing shows a blister, the other wing always has abnormal veination. In a study on the variation of the trait, individuals were classified as (1) slight; that is, with abnormal veination and no blister; (2) definite; that is, with one or both wings having a blister regardless of the abnormal veination. The expression of the character is usually more marked in the female than in the male. Males - total count: 984; slight 788; definite 196 (80.1%: 19.9%). Females - total count: 773; slight 155; definite 618 (20%: 80%).

Neuhaus, M. Triploid stock in Drosophila simulans.

In order to obtain new hybrid combinations between D. simulans and D. melanogaster it was necessary to have triploid females in D. simulans. For this purpose, yw females with attached X's were mated to sn males. Gray females were looked for in the F^1 generation. 8 gray females were detected among 5380 yw females. Two of them gave the following progeny: One female gave 6 yw ♀♀; 1 y ♀; 3 sn ♀♀; 4+ ♀♀; 3 yw ♂♂; 1 yw sn ♂; 3 sn ♂; 3+ ♂; 1 yw ♂; 1 w ♂. The other female gave 4+ ♀♀ and 1 w ♀. These data indicate the possible triploidy of these females. All gray daughters of the first female proved in further crosses to be diploid. One of the gray daughters of the second female gave 9+ ♀♀; 14 sn ♀♀; 2+ ♀♀ and 1 w ♂. It is obvious that this female was triploid. It was noted, simultaneously, that among these gray females there happen females with somewhat thickened scutellars and some other macrochaetae on the thorax, a part of which was forked-like. It was surmised that these females were triploid. This was confirmed in further experiments.

The data show the offspring of females with such bristles. Obtained crosses between females with thickened bristles and sn males - Females: + = 51; sn = 64; 3N = 38; y w = 4; w = 1; y = 1; Males: sn = 42; w = 3; y w = 2; + = 3; Intersexes + = 23; sn = 22. The cytological analysis proved this assumption to hold true. Ovaries of females with thickened bristles were stained with acetocarmine and their study showed the chromosome set to be triploid. Intersexes obtained in D. simulans differ but slightly from those in D. melanogaster. Triploid D. simulans females were mated to D. melanogaster sc^8 w^a f B males. The sc^8 chromosome was selected for the following reasons. It is known that the sc^8 - chromosome shows the Hw effect, which is manifested both in homo- and heterozygotes by the presence of a group of new microchaetae - mesosternale. Triploid females in D. melanogaster, having one sc^8 - chromosome do not manifest this character. It was therefore, possible to assume that these bristles would not appear in hybrids, having two chromosome sets from D. simulans and one set from D. melanogaster, which would enable us to detect them from diploid hybrids. Following hybrids were obtained: 5 $\underline{+\ +}$ ♀♀; 4 sn ♀♀; 2 + ♂♂; 2 sn ♂♂; 4 + ♀♂; 1 ♂ ♀. Although the figures obtained are small they nevertheless show that hybrids which have received from D. simulans two sets of chromosomes and the cytoplasm, and from D. melanogaster - one set of chromosomes probably very seldom survive. This fact is in conformance with previous data on hybrids between D. simulans and D. melanogaster. It is known that hybrid females having the cytoplasm and one X-chromosome from D. simulans and the other X-chromosome from D. melanogaster have a decreased viability. One case of a hybrid female having the cytoplasma and two sets of chromosomes from D. simulans and one set from D. melanogaster is, however, described in literature (T. Morgan, C. B. Bridges and J. Schultz 1938). The 5 hybrid intersexes obtained by us were similar to hybrid intersexes which were obtained from 3N melanogaster crossed to 2N simulans and described by Schultz and Dobzhansky (1933). Such hybrids were also obtained in great number by us.

Pipkin, Sarah Bedichek. Expression of forked in the progeny of triploids.

The degree of forkedness observed in the progeny of homozygous forked triploid females crossed with forked males is found to depend not only upon the dosage of the X-chromosome but also upon the dosage of the autosomes. Super-males (1X3A) are the most forked; triploid (3X3A) and diploid (2X2A) females next; diploid males (1X2A) less forked; and intersexes (2X3A) least forked of all. Forked haplo-IV diploid males with bristles as slender as those of the super-males nevertheless have a degree of forkedness similar to that found in diploid males. Intersexes, while less forked generally than diploid males, sometimes have patches of extremely forked tissue. Thus the autosomes influence the expression of forked since 1X2A diploid males are less forked than 1X3A super-males, and, furthermore, 2X2A diploid females are more forked than 2X3A intersexes. In X-chromosome aneuploid experiments concerned with studying sex balance, it was found that 3A individuals carrying one complete X containing forked, one complete X with the normal allel of forked, and the left hand X-chromosome fragment of an X-IV translocation broken between the loci of fw and wy, were faintly forked. Ordinary 3X3A triploid females with one dose of the normal allel of forked and two doses of forked appear non-forked. Control intersexes (2X3A) with one normal allel of forked and one forked gene present also appear non-forked. Thus although the number of each autosome remains the same (three of each), the addition of an extra fragment of the X not containing the locus of forked to the $\frac{+}{f}$ 3A complement changes the expression of forked. Individuals of the composition $\begin{array}{c}\rule{1cm}{0.4pt}\\ + \\ \hline f\end{array}$ 2A, (hyperdiploid females), however, only rarely show one or two bristles faintly forked in comparison with the weak but definite forking in nearly every bristle of practically every individual of the composition $\begin{array}{c}\underline{}\\ \underline{+}\\ \underline{f}\end{array}$ 3A. More data are now being accumulated in an effort to localize the region in the left hand portion of the X-chromosome which appears to be responsible for the weakening of dominance of the normal allel of forked in $\begin{array}{c}\underline{}\\ \underline{+}\\ \underline{f}\end{array}$ 3A aneuploids.

Pogossianz, H. E. The gene scute in D. virilis. Among 60,986 F^1 females, 46 scute flies were obtained from the cross of sc y sc v c females to normal males, given 4000 r of X-ray treatment. Simultaneously in melanogaster only 3 acute mutations occurred among 12, 858 F^1 females. Since the method and dosage in both experiments are the same and the allels of scute in females used are phenotypically similar, the following conclusion can be drawn: the gene scute in virilis mutates more frequently than in melanogaster. This is confirmed by the data obtained on melanogaster by other authors (Goldat 1936, Glembozky 1936). Among 46 scute mutations 16 were not tested; they were sterile or perished. The remaining 30 flies carried the newly arisen mutations. 18 of which proved to be viable and fertile, 4 sterile in males, and 8 lethals. All these mutations are studied at present both genetically and cytologically. The description of the new scute mutations is given in this issue of DIS.

Sirotina, M. I. Cytology of D. Busckii. An investigation of metaphase plates in larvae ganglions and in ovaries of a stock of D. busckii of Kiev origin showed the presence of only three pairs of chromosomes (instead of four pairs reported by Metz for the American D. busckii). The X-chromosome is rod-like with a sattelite on its proximal end. The autosomes are V-shaped, with equal arms; both pairs are alike in length. The Y-chromosome is likewise V-shaped, but one arm is longer than the other. An analysis of salivary gland nuclei revealed the absence of the granular amorphous central mass characteristic for D. melanogaster, and the presence of a heavily staining nucleolus. All the elements are connected with this nucleolus by thin threads. The number of elements is 6 in the female and 7 in the male; the extra element in the male is the Y-chromosome (or its part), containing about 14 discs. The sattelite of the X-chromosome is likewise represented in salivary nuclei as a free element. The X-chromosome and the sattelite are more strongly connected with the nucleolus than all the other elements. A rather detailed map of the salivary chromosomes of this species has been prepared and will appear in the paper which is now being prepared for press.

Serebrovskaja, R. I. X-ray induced mutations in D. hydei. By means of X-rays (3000 and 4000) the following mutations were obtained in D. hydei: (1) scute - sex-linked, recessive (2) white - sex-linked, recessive (3) vermilion - sex-linked, recessive (4) forked - sex-linked, recessive (5) Notch - sex-linked, dominant (lost) (6) orange eyes - sex-linked, recessive (7) red eyes - sex-linked, recessive (8) miniature - sex-linked, recessive (lost) (9) Dichaete-type - autosomal dominant (lost) (10) Spread-type - autosomal dominant (lost). In total, 32751 flies were examined. Both sexes were studied and X-rayed simultaneously. The greatest attention in our work was paid to the scute mutations. Our allelomorph (scute^{2}) affects a great number of bristles, such as : all the 4 scutellars, the praesuturals, postalars anterior and posterior, supraalars ant. and post., sternopleurals - ant. and post., orbitals 1-3, verticals 2, intraocellars, mesosternals, vibrissae, genals, mentals, coxals 1,2,3, costals dorsal proximal, costals 1 and partly sternitals. When compared to the scute allelomorphs of melanogaster the scute of D. hydei is more proximate to the group of scuti longi which reduces the bristles of the B, C and D blocks (see the communications of A. S. Serebrovsky in this issue). It is of interest to note the influence of scute^{2} upon the bristles of the genitalia, a fact never observed in melanogaster, due probably to a different structure of genitalia.

Serebrovsky, A. S. Further study on scute allelomorphs. A thorough study of a considerably greater number of bristles, controlled by the gene scute, has allowed us to divide all the bristles into four groups (blocks); the A-block, controlled by the achaete allelomorphs, and the B, C and D-blocks, controlled by the scute allelomorphs, Block A: involves the bristles: dorsocentrals, "thoracals", "trapezals", "coslals basal", "antennals external", femorals 3 ventral", interocellars, "antennals basal", subcoxals", microchaete sternopleurals, "ciliars", microgenals", frontocentrals, " femorals I, 2 and 4", "annulars", verticals I. Block B: involves the bristles: notopleurals I, praesuturals, "femorals I 1 and 3", mentals", sternopleurals an. and post., coxals, orbitals, postverticals, ocellars, postalars ant. (?), vibrissae (?), verticals (?). Block C: involves the bristles: scutellars, sternitals, "tergitals", "genitals" (?). Block D: involves the bristles: humerals, postalars post., verticals, supraalars ant. and post., notopleurals - 2. The allelomorphs of scute fall into three groups: scuti brevi, scuti medii and scuti longi. Scuti brevi (sc^{5}, sc^{4sh}, sc^{2}) affect in usual laboratory conditions the block C; scuti medii (sc^{1}, sc^{7}, sc^{9}, sc^{B1}, sc^{29}, sc^{2sh}, sc^{I7}) the blocks B and C. sc^{6}, affecting the block B can also be included in the latter group. Scuti longi (sc^{L8}, sc^{s1}, sc^{4}) affect the block B, C and D. The longest, scute^{3}, affects all the blocks (A, B, C and D) simultaneously, thus including both scute and achaete. Finally sc^{A1} links scute and achaete, affecting the block A and B, as well as sc^{I3}($sc^{1} + ac^{3}$). To judge from the data of Pogossianz, Varshaver and Serebrovskaja analogous types of allelomorphs exist in D. virilis, simulans and hydei.

Shapiro, N. I. The rate of spontaneous sterile mutation. The frequency of sterile mutants, functioning in females, was studied. Recessive steriles, arising in the 2nd chromosome, were registered. The method used in the experiment prevented from mixing the newly arisen steriles with those which had been previously in the population. Among 2,841 chromosomes studied, one sterile was detected. In the same experiment 18 newly arisen lethals were detected among 3,132 chromosomes. The data obtained indicate a considerably lower frequency of spontaneous autosomal sterile mutation as compared to the lethal mutation rate.

Steinberg, Arthur G. Growth curve of Bar and wild type eye discs. Using the technique described by Medvedev the growth curves for Bar and wild type eye discs were measured. Measurements were then taken at twelve hour intervals from thirty-six hours after hatching until puparium formation. The experiments were run at 27 ± 1° C.

The data show that the Bar eye discs are already smaller than wild at 36 hours after hatching and that the growth rate of both Bar and wild type is the same throughout this period of development. These data are especially interesting in view of the fact that the temperature-effective period is included in this time interval.

Steinberg, Arthur G. The Lobe alleles and the v^+ hormone.

Implantation of eye discs from larvae which are genotypically Lobe, $Lobe^2$, $Lobe^4$ or $Lobe^5$ into vermilion hosts shows that such discs fail to develop wild type pigmentation. Their pigmentation is intermediate between that of vermilion and wild type. This reaction is similar to that shown by the Bar "alleles".

Steinberg, Arthur G. Facet number of Bar^4

The facet number of B^4 ♂♂ and ♀♀ at 25° C has been determined. The ♂♂ have 560.0 facets and the ♀♀ 558.2.

Timofeeff-Ressovsky, N. W. Determination of the "radius of activity" of Drosophila flies.

For many theoretical considerations the knowledge of the real amount of panmixy or, vice-versa, of isolation within the species-populations is rather important. Without considering some finer mechanisms of physiological and ecological isolation, three main factors are of importance in this connection: (1) the real distribution of individuals over larger areas within the different parts of the species-population, (2) the "radious of activity" of the individuals within one generations, and (3) the extension of "life-waves" (quantitative fluctuations in time and space) in populations, and of accidental, passive mechanisms of mixture between different parts of a larger population. The relation of the first two factors can show the amount of "active" panmixy, and the knowledge of the third factor can give an idea of the amount of "passive" panmixy. More or less systematical, extensive, and exact studies on the "radius of activity" were so far made only in birds; they showed tremendous dissipation of the brood in each generation in some species (e.g. Nettion crecca L.), and extraordinary territorial conservatism in others (e.g. Sturnus vulgaris L.). The following simple method can be used in studying the "radius of activity" in Drosophila: A ground of the size of about 2 - 5 hectars is divided into equal squares (on the map!) and in the center of each square (10 - 15 m apart) a bottle with food is placed; in the middle of the ground larger amounts of food are placed, and 2000 - 5000 Drosophila flies with different (not too deleterious!) mutations (better - combinations of 2 - 3 mutations) as "markers" are let out. During a period of 15 days the food bottles are inspected twice a day (9^h and 18^h), and the "marked" flies are counted, registered, and let out at the same place where they were caught (or collcted and killed!). The end-result of a 15 days experiment will show the "dissipation-area", or "radius of activity" of the individuals of one generation of the species in question. Such tests have as far shown that the diameter of the area where "marked" flies are caught (after they were let out in the center of this area) is about 100 - 200 m, differing according to the species and mutations used, and also to the meteorological conditions. D. funebris shows, so far, a higher "dissipation" than melanogaster. The same type of experiment can be modified: instead of imago-flies, "marked" larvae and pupae can be placed (with a supply of food) in the center of the "experimental field".

Timofeeff-Ressovsky, N.W., H. Boehm and G. Gottschewski. Studies of population-structure in Drosophila.

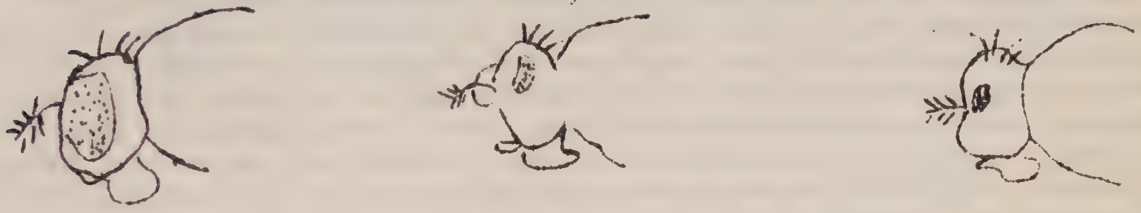
For theoretical considerations on the gene-dynamics in free-living populations and on micro-evolutionary processes, we need some knowledge of the real distribution in time and space of the individuals within different parts of the distribution-area of the species. The following simple method of studying the population-structure in Drosophila can be used: A ground about 5 - 15 hectars large is divided into equal squares (on the map!) and in the center of each square (15 - 25 m apart) a bottle with well-fermentating food is placed. The squares and respective bottles are serially numbered on a map. The Drosophila flies are counted and registered (according to species and square) 2 - 4 times a day for a period of 3 - 4 days. Such registrations should be repeated every 3 - 4 weeks during the whole season. The result will show the actual distribution of individuals of different species in time and space throughout the season. From time to time countings should be made during 24 hours, day and night, every 2-3 hours; they will show the activity-curve of the flies for 24 hours. Any meteorological, phytosociological, and ecological data should be collected and used in the evaluation of the results. The results so far obtained at three places in this country show that: (1) D. melanogaster builds small but dense populations more or less far apart from each other, and does not occur in between; the obscura group of species, as well as such species like transversa or phalerata, are distributed much more regularly throughout the suitable biotops; the distribution of funebris is more like that of melanogaster, but shows more dissipation around the single dense populations (2) The distribution of caught individuals throughout 24 hours shows two marked peaks in the morning (8 - 10^h) and in the evening (18^h), and "dead" periods in the night ($0-4^h$) and at noon (13 - 14^h) (3) The first flies appearing in spring are those of the obscura group, which then occur regularly throughout the whole season; funebris appears in larger quantities in May-June, and melanogaster, transversa, and phalerata - later, in July (4) The flies of the obscura group show high activity several hours after rainfalls; melanogaster disappears (is not caught in the food bottles) in cooler days and weeks.

Tiniakoff, G. G. The "Bar and "aristapedia" mutations in D. funebris.

The dominant sex-linked "Bar" mutation has been obtained in F^1 from X-rayed males with the "mottled" mutation which represents a reciprocal translocation between the Y and the IV chromosomes (See schemat. cytolog. maps in the work of G. G. Tiniakoff in the Russian Biological Journal, vol. V, 1936, p.754). The phenotype of the bar mutation, similarly to that of D. melanogaster, is expressed in a greatly reduced (see Fig. 1), striated eye. The expression is much stronger in males than in females, but a few facets remaining sometimes in the former. Bar males are less viable and fertile as compared with females. According to preliminary data, only about 17 per cent of bar males are obtained from a cross of bar females to normal males. When crossing bar females to bar males, the strain obtained shows poor development, no homozygous bar females being, as it seems, produced. A cross of bar males to normal females gives offspring where all females are bar and all males normal. It was shown cytologically, that this bar strain represented an insertion of a rather large section of the median part of the X-chromosome into the 2 chromosome (See cytolog. maps of G. G. Tiniakoff). The dominant autosomal "aristapedia" mutation was obtained in F^1 by means of X-raying normal males from the "Polivanov" strain of the Moscow district.

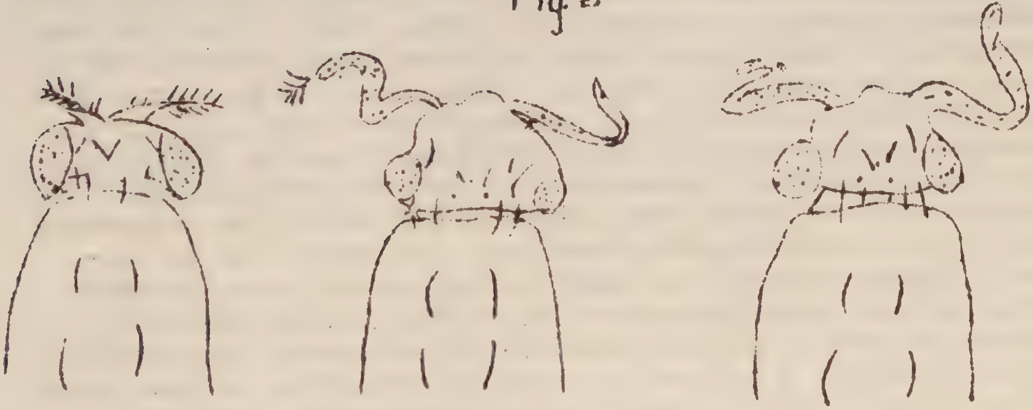
The phenotype of the "aristapedia" mutation consists in a gigantic growth of these segments of the antenna on which aristae are disposed. In flies with the above character well expressed, antennae are transformed into legs with a division into segments proper to them (Fog. 2). Large bristles, characteristic of legs, are sometimes seen to grow between the segments (see the extreme right Fig. 2). On the ends of those atavistic legs, well marked aristae are occasionally present. The head of flies with the aristapedia mutation is somewhat elongated, while the eyes are always reduced, flattened, resembling in form the "lobe" mutation in D. melanogaster. The manifestation of that character is found to be more pronounced in females than in males. Cytologically this strain has not yet been investigated. Among 120,000 F^1 flies examined, resulting from X-raying males of diverse strains, the bar mutation was obtained only once and the aristapedia mutation only twice, the latter character occuring in both cases in males. The second male did not produce any offspring. As far as we know, the bar mutation and the atavistic mutant aristapedia character in Drosophila is a very rare phenomenon and our cases in D. funebris seem to be described for the first time. The recessive aristapedia character in D. melanogaster was also described in the Institute of Experimental Biology by Balkashina in 1928.

Fig. 1



Norm. Bar ♀ Bar ♂

Fig. 2



orm. Aristopedia ♂ Aristopedia ♀

Varshaver, N. B. Mutation rate of y, ac, sc, w and sn in D. simulans.

Normal D. simulans males were X-rayed (dosage 4000 r) and mated to y w females with attached X-chromosomes. The following frequency of mutation was detected in the loci y, ac, sc, w and sn. Among 94,762 studied F^1 males there were found: 11 yellow (0.0116%), 0 achaete, 10 scute (0.0105%), 26 w (0.0274%), 1 mottled

(0.0011%) and 12 singed (0.0126%). Among the 10 scute mutations only 3 proved to be fertile, although showed a very poor viability. One of the latter was lethal in females, just as the sc^3 in melanogaster. None of the allelomorphs studied was connected with a long inversion. The description of scute mutations in simulans showed their high resemblance with the scute in malanogaster.

Technical Notes

Clancy, C. W. Large scale egg collections.

In order to secure large numbers of pupae of known ages it has been necessary to develop special methods for handling the females and for collecting eggs, the details of which may be of interest to other workers. (A) Pre-feeding of females. The importance of pre-feeding the females for egg-laying was mentioned in a previous issue (W. P. Spencer, DIS-8). The technique adopted here is to start culture bottles of the desired stock with 5 to 10 young females and an equal number of males. Allow the females to lay for 2 to 3 days, then transfer to anew bottle; repeat several times. The females will usually lay well for a week to ten days; it is then best to start bottles with new ones. Dried Brewer's Yeast in rather heavy suspension is added to the culture bottles a day or two after the females have been transferred from them. This ensures maximally fed larvae. When cultured as indicated the flies are large and the females after being counted, mated and fattened for about two days in regular culture bottles to which a small amount of water has been added, are in excellent condition for laying. For the ageing, mating, and fattening process, 50 to 70 females are placed in a bottle with 25 to 30 males. These are later transferred without etherization to the egg collecting jars described below. (B) Collection of eggs. Eggs are collected in quart size, wide-mouthed, fruit jars, (Presto, manufactured by Owens-Illinois Glass Company) in small metal trays containing agar-cornmeal-molasses medium. The trays may be purchased at Woolworth's under the name of egg-poaching pans. They are made of aluminum and consist of a pair of small trays 7x7x3 cm. deep, connected by a flat metal bar. A wire handle is attached to the middle of the bar. This is punched out and the connecting piece cut across the middle, giving two pans, each bearing asmall tongue that is convenient to use as a handle in transferring to and from jars. Permanent plugs for the jars are made by stuffing heavy gauze or muslin with coarse cotton and tying with heavy thread. They can be autoclaved with dry steam. To ensure a humid egg-laying surface (Spencer, DIS-8), the constant temperature room, in which the egg collections are made, is kept at 70% to 80% relative humidity; the food used in the pans is diluted one-third to reduce the agar content to about 1%; and, finally, 2 to 3 cc. of a watery suspension of live yeast is pipetted on the food surface. Yeast or its fermentation products seem to be important not as a means of eliciting the ovipositing reaction, but rather as a source of something used by the females for continued egg production. Pre-fed females lay well for several days on moist trays without yeast, but their egg production soon drops off, while comparable flies given plenty of yeast usually continue to lay heavily for 10 to 14 days. No experiments have been made to test this observation, but experience indicates that in some way yeast is necessary to maintain high egg production.

DeMarinis, Frank. An apparatus for facilitating direct facet-counting.

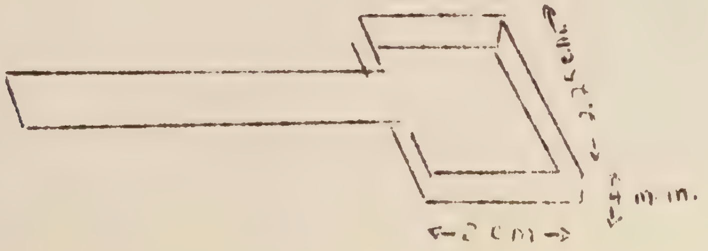
A simple and convenient apparatus can be easily constructed which will facilitate direct facet-counting or other somatic examination of Drosophila. A smooth steel ball, 2 cms. in diameter fitted on a metalic socket, 1 cm. high, 2 1/2 cms. external diameter and 1 1/2 cms. internal diameter is all that is required. By placing a very small amount of glue at the top of the ball the specimen can be made to stick conveniently for examination. With a simple rotation of the ball with the fingers the observation may be made at any point in the curvature of the eye or the body, as the case may be.

Dempster, E. R. Shipping flies in cold storage.

At the suggestion of Dr. W. L. Waterhouse of Sidney, Australis, we made two shipments of several stocks of Drosophila melanogaster to Australia in the summers of 1937 and 1938 in the chill room of Matson Line steamers, hoping thus to successfully evade the lethal equatorial temperatures. The Matson Co. has given us the following values as temperature limits: 38° F (3-1/3° C) to 40° F (4-4/9° C). The flies remained in the chill room approximately 4 weeks in each case. The shipment of 1937 was completely successful, that of 1938 a complete failure. In both cases flies were introduced into 1" x 6" test tubes containing approximately 1-1/2" of food and stoppered with extra-tight fitting gauze and cotton stoppers, well wrapped to prevent evaporation and with no holes thru the packages for ventilation. Our experience in general, especially for airplane transfers, has indicated that evaporation is a much more serious hazard than lack of ventilation. The flies were kept at 25° C for approximately 8 days before being placed in the chill room with the hope that some individuals in a relatively resistant stage of development would at least survive. In the 1938 (unsuccessful) shipment the standard cornmeal-molasses formula (Bridges, DIS-6:27), to which was added about .07% Moldex A, was used. In the 1937 (successful) shipment the medium was approximately the same except that only 1% of agar was used and about .75% of cooked dried yeast added. Very likely the failure in 1938 was due to the use of too dry a medium; probably a very wet medium would be best for such low temperatures.

Sidky, A. R. New spoon for egg counting, and method for seeding.

While studying egg-laying in Drosophila a new spoon for egg counting was constructed and used instead of the ice cream paper spoon commonly used. It proved very satisfactory. The spoon is made of aluminium of about 0.5 mm. thick. Its shape and dimensions are shown in the illustration. The width of the spoon is so planned



that it just fits the vials used. The 150 ordered by the Institute were supplied at one penny each. The food is poured into spoons in a liquid state through a funnel fitted with a rubber tube and aclamp. The food takes about one minute to harden. A large number of spoons can be prepared in a very short time so that a few days' supply can be prepared and kept in a refrigerator. This spoon is superior to the paper one in the following respects: (1) The main advantage is that, the surface being level, one can count all the eggs with one focussing and so save much time. (2) The food is of uniform thickness and the edges do not get dry and make counting or collecting the eggs difficult. (3) The food does not stick to the spoon, and when it is to be removed for incubation, etc., it can be lifted completely out of the spoon by merely inserting the point of a blade or a needle. No eggs are lost or crushed in the process. (4) Being made of aluminum it is very durable, stands any amount of boiling and sterilizing, and is very easily cleaned. It does away with the recoating with paraffin which is necessary with the paper spoons. The counting is done by marking the surface of the food with a needle and so dividing it into two or three rectangular areas according to the field of the binocular, and passing the spoon backwards and forwards while counting. Instead of the usual method of allowing a drop of yeast to fall on the food, which results in the yeast growing into a large lump containing many eggs and so renders the counting difficult, painting the surface of the food with a thin suspension of yeast using an ordinary small camel hair brush thus providing a uniform thin film, of yeast, gave very good results.

Slizynska, Helen. The method for obtaining any number of virgin females.

Since the sex in Drosophila can easily be determined at the larvae stage, the way of collecting virgin females is based upon the separation of females larvae from the males. The female flies gathered in this way are beyond any doubt virgin.

Nomenclature

(See also Muller's comments on pages 39-43 of this issue about nomenclature section of DIS-9)

Mohr, Otto L. Nomenclature and symbols. For those who are not familiar with the Drosophila system of symbols through personal work, the fact that there is in Bridges' system no distinction between symbols for genes and aberrations with *phenotypic* effects and symbols designating the nature and origin of structural changes without visible effects, is apt to cause confusion. - This may easily be done away with in the following way: Symbols for genes and for the phenotypic effect of genes and of aberrations should be written in italics (e.g. w^e, *D*, *N*). Symbols for structural changes should be printed in Latin letters (e.g. T(3;Y), In(1)dl-49). Hence for instance Inversion 3L Payne with gleam which be Bridges is denoted In(3L)P^{gm} would receive the formula In(3L)P *gm* and the third chromosome deficiency which causes the dominant character complex Vein andwhich by Bridges is denoted Df(3)Vn, should receive the formula Df(3)*Vn*, etc. This enables the reader to differentiate between the two different categories of symbols. - It may perhaps be difficult to introduce this change in the typed DIS numbers but it ought to be stated in a foreword that authors are requested to use this system in their printed publications. - Further, in Bridges' system the basic locus symbol for a series of multiple allels originates from the designation of the first member of the series discovered. Thus in the Henna series the first member was dominant and was accordingly given the symbol Hn. Two later allels are denoted as Hn^r and Hn^{r2}, where r stands for recessive. This system is very cumbersome. Dominant members of allelic series are rare. When recessive members occur it is much more practical to use the symbol for the recessive allel as the basic locus symbol and add the superscript *D* for the dominant member (eventually *Da*, *Db*, *Dc* if additional dominant members should turn up). Thus the henna series would be hn^D, *hn*, hn^2 instead of Hn, Hn^r, Hn^{r2}. - It may be added that both these suggestions were discussed at the conference on terminology held in London before the last congress and received unanimous support.

Research Notes

Argelander-Rose, A. Experiments with hemithorax. In November 1937 about a dozen vg^{hem} flies appeared in several cultures of vg ar sp. Manifestation at 25° differed between 0 and 7%; in the average of more than 6000 flies it was 1.62%, 2.53% in females and 0.60% in males. After crossing this stock with x-ple, b cn vg, al dp, and L^2/Cy manifestation increased in some cultures up to 25% without, however, being constant. In a stock b pr vg it was then kept at an average of 9.3%, 11.2% in females and 7.4% in males. vg^{hem} flies mostly display absence of the right or left dorsal half of the thorax, very seldom the whole thorax was found missing. Sometimes the thorax was split in the midline. Also a great number of flies were found with a protuberance at one or both sides of the thorax (vg^{hem} P). Among 143 flies 62.2% were vg^{hem} and 37.8% vg^{hem} P. vg^{hem} and vg^{hem} P could not be selected. In stocks where vg^{hem} appears a large number of flies with extreme vg, wings reduced to knobs or very short, can also be found. - In some cultures of our stock which were left in the room at about 18-20° 50 to 70% vg^{hem} and vg^{hem} P arose, the next generation, however, bred at 25°, gave again only about 10%. A preliminary experiment showed an influence of temperature on the amount of manifestation. The time of development from mating the newly hatched parents until F_1 began to hatch was 10.1 ± 1.0 days at 25° and 22.1 ± 1.6 days at 15-18°. Cultures which were kept at 15-18° for the first three days andthen transferred to 25° took 11.6 ± 0.7 days, those which stayed at 15-18° for four days and in 25° thereafter needed 11.6 ± 0.5 days. Fertility is 81.6 flies per vial at 25° and 49.8 at 15-18°. Manifestation in the offspring of the same parents, brought subsequently into different temperature, was as follows:

Temperature	Number of cultures	n	vg^{hem} %	$vg^{hem\ p}$ %	Total vg^{hem} %
25°	20	1633	9.6	3.4	13.0 ± 6.4
15-18°	34	1694	20.8	10.4	31.2 ± 14.8
3 days 15-18°	14	262	13.4	6.5	19.9
4 days 15-18°	20	635	11.0	6.6	17.6

Manifestation is higher among the early hatching flies, particularly in mass cultures. At 25° 19.8% of the flies hatching on the first and second day were vg^{hem}, on the third and fourth day 11.3% appeared and on the fifth to ninth day 3.9%. In 15-18° the respective numbers were 45.7%, 40,3% and 32.6%.

Argelander-Rose, A.
Fertility of ClB females.

After one single copulation ClB/sc^1 - and ClB/y cv v - ♀♀, bred at 25°, gave an offspring on the average of 196.2 ± 25.6 flies. Fertilized eggs were laid during 9.8 days. The offspring hatched as follows on the

1st	2nd	3rd	4th	5th	6th	7th	8th	9th	day
26.7	20.2	22.2	14.0	5.8	5.5	2.0	2.2	1.4	%

When mated to the males for three days the average number o the offspring was 138.4 ± 65.8.

Argelander-Rose, A.
Experiments on motor activity.

The flies, melanogaster, were put into a glass tube, 30 cm high and 4 cm wide, and by shaking the tube brought to the bottom. Then the time the fly needed to run to the top was taken. With each fly 5 experiments were made, one after the other, and that repeated on several days. In the average of 40 ♂♂ and 25 virgin ♀♀ (Berlin wild, w sn^3 B, w^e and y pn) the time for the way of 30 cm was 30.5 ± 8.5 seconds for the males and 45.0 ± 6.3 seconds for the females. No difference was detected with respect to age (1-14 days) or daytime (11.30^h and 19^h) and no improvement within 6 days. An attempt is being made to select fast and slow flies.

Argelander-Rose, A. A case of high non-disjunction.

In a cross between ClB v/w sn^3/Y ♀ x y cv v ♂ one female appeared with v eyes which proved to be w v/y cv v/Y. In F_1 to F_3 the exceptional v females mated to wild stock males gave 2.9% exceptions, 13 ♀♀ and 7 ♂♂. In F_4 one culture gave 36.5% exceptions among 72 flies. From the exceptional v females of this stock cultures were obtained with an average of 22.3% exceptions (type A) as well as with 1.6% (type B). The cause for the high non-disjunction lies in the w v-chromosome. Of the regular females half are expected to be XXY-♀♀. From 61 regular w v/++ and w v/++/Y♀♀ 11 cultures of type A with 20.1 ± 7.7% exceptions were obtained, 20 cultures of type B with 3.5 ± 2.6% and 30 cultures with no exceptions where the mother had been and XX-♀. On the other hand, 18 regular y cv v/+++ and y cv v/+++/Y females gave no type A, but 10 cultures of type B with 1.9±1.3% exceptions and 8 cultures from XX-mothers. In the course of the experiments 5 equational exceptions of the constitution w v/w v/Y and w cv v/w v/Y were found which gave the same amount of exceptions in type A and B. The offspring of 365 exceptional females carrying the w v-chromosome and originating from mothers of type A showed 180 type A and 190 type B, whereas exceptional females coming from cultures of type B gave no type A at all. In the average of 137 type A cultures the exceptions amount to 18.7±6.2% and 180 type B cultures gave 3.5±2.6%. Crossing-over in the w v-chromosome is very much reduced with type A. Instead of 13.7% w v/y cv v/Y females gave on the average 1.4% crossing-over in type A and 7.1% in type B. The assumption is made that a duplicated piece of the w v-chromosome translocated to one of the autosomes might be responsible for the ghigh non-disjunction in type A. That would also explain the fact that type A splits up into A and B. Cytological proof of this assumption is about to be sought.

Bishop, Maydelle.
Bar-263-48.

A female with Bar-like eyes (experiment number 263-48) was found among the offspring of a cross between X-rayed (3000r) Swedish-b males and y pn females. This was crossed to y pn males and the offspring were recovered in the following proportion: B-like: females 17, males 11; y pn: females 11, males 12, which indicates that rearrangement in the X-chromosome might be involved. The mutant males proved to be fertile, and salivaries of their daughters were analyzed. This analysis showed a section from 16A into the chromocenter region (20) is transposed in the normal order into 3E. Female offspring of the mutant males crossed with the heterozygous mutant females show great variation in the amount of reduction of the eye, but no distinct division into two classes could be detected. Individual mating of females showing the greatest reduction yield both mutant and y pn males, indicating that homozygous mutant females are usually or probably always lethal. The eye-reduction is about half-way between B and B^1 and varies little in the male. - Description: B^{263-48} - Bar-263-48. Bishop, September 26,1939. Tp. From X-rayed Sw-b ♂. Eye-reduction in ♂ and het. ♀ between B and B^1; size constant in ♂♂ but variable in ♀♀ . Hom. ♀ lethal, ♂ fertile. Salivaries show a segment with a brea in 16A and the middle of 20 inserted into 3E in normal order. RK2A.

Boyd, M.M.M. and H. Spurway.
Over 50% crossing-over between two loci in D. subobscura.

The F_2 progeny from scarlet x interrupted were 95++; 27s+; 38+int; 00 s int. Therefore the loci are on the same chromosome. - Nineteen cultures have been counted in the test crosses using these loci. In 13 of these the number of crossovers between the two is greater than the number of non-crossovers, and out of the total 2,295 flies examined, 1,208 are crossovers, i.e., the recombination percentage is 52.6±1.0. - In 7 of the 19 cultures the probability of obtaining as large or larger a deviation by chance from the expected 1:1 in the single factor ratios of one or both mutants is less than 5%. As the recombination percentage calculated from the remaining 12 cultures is 52.4±1.3 the anomalous proportion of crossovers does not seem to be due to the abnormal segregations of the individual mutants. - More figures are being collected of the two point crosses to reduce the standard error and multiple stocks are being built up for examination of crossing-over and interference in the region between the 2 loci.

Braun, W. An exceptional case of factor dissociation in Drosophila melanogaster.

In a series of experiments which were designed to test the production of mosaics after X-radiation of the father, normal males were irradiated and then crossed to y w^a ec cv ct v m g f/ClB females. Among 220 crosses of that kind many exceptional males were observed in the F_1 of 3 crosses which had been started at the same time. Culture No. 1783 contained ♀♀ : 66 normals, 6 ClB; ♂♂: 6 y - f, 6 normals, 2 f (single crossover), 1 g f (single crossover), 1 y - g (single crossover), 1 y - cv (single crossover), 1 y w^a (single crossover), 1 g (double crossover), 1 y m g f (double crossover), 1 w^a m (triple crossover), 1 w^a m g f (triple crossover), 1 y ec g (4-ple crossover), 1 y w^a ct m (4-ple crossover), 1 y ec cv ct g (5-ple crossover) and 2 w^a ec cv ct m f (5-ple crossover). Culture No. 1782 ♀♀ : 31 normals, 11 ClB; ♂♂: 4 y - f, 1 normal, 1 f (single crossover), 1 w^a m f (5-ple crossover). Culture No. 1780 ♀♀: 8 normals, 3 ClB, 1 y - f, 1 w^a m ct, 1 w^a g f, 2 w^a m ct cv, 1 w^a f, 1 m g f, 2 w^a ct m f, 1 f; ♂♂: 3 y - f, 5 normals, 2 g f (single crossover), 1 y w^a ec (single crossover), 1 v m g f (single crossover), 1 w^a cv ct m (5-ple crossover), 1 y ec g (5-ple crossover). - We will not discuss the production of the exceptional females of Culture No. 1780 here, because their appearance is due to changes produced by X-radiation of the father. The appearance of the exceptional males in these 3 crosses, however, presents a puzzling problem. It has to be noted that, (1) although ClB females are produced, none of the exceptional males shows B (this excludes the possibility of a loss of Cl in the mother), (2) the exceptional males represent single, double, triple, 4-ple and 5-ple crossover classes. (3) When the exceptional males were mated to y or y-f/ClB females they behaved like ordinary crossover males. Three explanations can be suggested for the production of these exceptional males. (1) ClB is known to exhibit non-disjunction rather frequently. The mother may have been a XXY female and exceptional crossovers between X and Y may have taken place. The very rare occurence of reciprocal crossover classes in the exceptional males may indicate such an exceptional process. (2) The lethal which normally is located at the left break of the inversion may have disappeared and a lethal close to B may have been present. In such a case an exceptional great number of crossovers may have taken place into the inversion and the classes which receive B would receive the lethal too, therefore no B males among the exceptional males. The great number of double and triple crossovers may indicate that the crossovers took place into the inversion. (3) The inversion may have been reinverted in some gametes and the B disappeared at the same time. - It was felt desirable to put this case on record here until a final solution can be found at some time. The writer would be pleased to learn of similar cases and to receive suggestions as to possible solutions.

Brehme, K. S. The Minute condition as a possible effect of a hormone deficiency.

It has been shown (Beadle and others) that v^+ and cn^+ hormones can be utilized by larvae when administered in food, in the form of boiled and crushed bodies of larvae which contain these hormones. In order to determine whether the Minute phenotype results from lack of a hormone present in the wild type, Minute larvae were fed on wild type larval tissue. Food was prepared as follows: wild type larvae were cultured under conditions of optimal feeding; at 84 hours from hatching, they were boiled in distilled water and mashed. Brewer's yeast was added to the amount of 1 1/2% of the weight of the larvae. The mixture was placed with moist filter paper in 4x1 inch shell vials, stoppered with cotton and autoclaved. Eggs from a mass mating of Mw/ca ♂♂

by ca/ca ♀♀ were collected on autoclaved egg spoons (method of Clancy) over a period of 5 hours; they were then immersed in 85% alcohol for 10 minutes and placed in the vials under sterile conditions. Of 30 to 40 placed in each vial, about 15 hatched. The larvae were observed to ingest the boiled larval tissue. At 8-hour intervals, puparia were removed from the experimental vials and placed on moist filter paper in clean vials. The experiment was conducted at 25° C. - Minute bristles and lengthened larval period were taken as criteria of the Minute condition. Of the 82 imagoes obtained, 41 had wild type bristles and claret eyes, 41 had Minute bristles and wild type eyes, the phenotypes expected if the Minute condition were not eliminated by administration of a hormone present in the wild type and deficient in the Minute. The mean length of the larval period of the wild type was 174 hours; of the Minutes, 203 hours. Puparium formation was retarded in both groups, probably due to inadequency of the food. Normally the average difference in pupation time of wild type and Minutes is 42 hours; this difference was greatly lessened under the experimental conditions. However, the Minute larval period is still significantly longer than wild type. - It may be concluded that the Minute phenotype does not result from deficiency of a hormone which is present in the wild type and can be administered by feeding. This result is to be expected from the evidence of somatic mosaics.

Brehme, K.S. Development of eye color in Minutes.

Beadle (1938) reported a change which occurs in the larval period 70 hours after oviposition; larvae removed from food before 70 hours die without pupating, those removed after this time pupate successfully. There is a period just before 70 hours when larvae are sensitive to low food levels; v larvae underfed at this time pupate a day or more later than their normal sibs and produce a small quantity of v + substance. Beadle's hypothesis is that metabolism is altered in starved larvae in such a way as to produce metabolites which are used in manufacture of v + substance. To examine the possibility that in normally fed v larvae a sub-threshold concentration of v + substance is formed and in starved larvae prolongation of the larval period allows time for production of an effective quantity of hormone, Brehme has used Minutes as an agent for prolonging the larval period. Fully fed Minute and non-Minute offspring from the following crosses were compared: Mw/+; bw/bw; v ♂ by bw/bw; v/v ♀ MFla/+; bw/bw; v ♂ by bw/bw; v/v ♀ , bw/bw; v ♂ bt Ml^2/bw; v/v ♀ , bw/bw; v ♂ by Ml/bw; v/v ♀ (Minute - brown crossovers were examined in the last two crosses). All flies had colorless eyes. This supports Beadle's hypothesis that a factor other than mere prolongation of development results in production of v + substance in starved larvae. It has also been found, by complete starvation, that the 72-hour change occurs 14 hours later in Mw (larval period prolonged 42 hours) than in wild type larvae (Florida stock); the change is 6 hours later in Ml^2 (larval delay of 12 hours) than in wild type (Oregon-R-C).

Bryson, V. Unspiraled testes in Drosophila melanogaster.

In the course of experiments to determine the modifying action of extreme Minutes, 94 males of the following genotype were obtained: l(3)39a M^w/Lyra. Of these, 18 showed little or no trace of the genital arch or anal plate, and the anus was imperforate resulting in complete closure of the intestine. Dissection revealed that in such males the testes were either free in the abdominal cavity or attached loosely to the intestine, in either case being oval in shape. The genital ducts were undifferentiated. This stock was maintained for five months, during which time the penetrance of the trait gradually fell off, presumably through the constant selection of fertile males.

Eloff, G. Effects of ultra-violet radiation on crossing-over between y and w in Drosophila melanogaster. Data so far obtained and involving 75 control and 52 experimental cultures comprising approximately 20,000 flies, indicate that 60 minutes irradiation of heterozygous female pupae by means of a Hanovia Quartz mercury vapour lamp caused a rise in the crossing-over percentage between the genes for yellow and white. However, both controls and experimental cultures yielded high crossing-over values viz. 2.18 and 2.95 which might be due to a slight rise in temperature on the stage of thequartz lamp. - An interesting phenomenon was the high percentage of wing abnormalities of the flies which emerge from the irradiated pupae. For the controls window-glass screens, and for the experimental material vitreosil plate of the same thickness were used. Screens prepared from the wings of the South African locust, Nomadacris septemfasciata, were used to gauge the penetrability of ultra-violet rays through chitin. It was found by spectrographic analysis that a vitreosil plate and screens of 3 wing thickness absorb the wavelengths shorter than 3650° A. Also no wing abnormalities were obtained with screens of 3 wing thickness, whereas 2- and 1-wing screens had correspondingly higher effects. - Another phenomenon observed was that among the y-w crossovers of both controls and experimental material at least 3 shades of red eyes were noticed in males and females. The reason for this is not yet clear. The experiments are continued.

Eloff, G. Mode of attachment of nucleolus to chromocenter in salivary gland nuclei of some Transvaal Drosophilids. Observations on salivary gland nuclei, especially of Zaprionus and of Drosophila funebris seem to indicate in several cases: (1) thepresence of radial lines of stress on the nucleolus; (2) a conical elevation on the nucleolus probably where the thread was attached; (3) slightly trumpet-like extremities of the connecting thread. These suggest that the connecting thread is of tubular nature.

Poulson, D. F. Effects of Notch deficiencies. The following Notches have been investigated embryologically to determine the nature of the developmental upset which prevents the appearance of Notch males: N 264-38a, N-8 (Mohr), N 264-19a, N 264-8a, N 264-40, N 264-47, and N 264-34. The embryological upsets are the same in all cases indicating that the locus involved lies within the facet band. - Only at the time of formation of the nervous system does development deviate visibly from normal. There is no separation into superficial cells and neuroblasts, and practically all of the ventral ectoderm becomes part of an abnormal nervous system. This leaves the ventral side without hypoderm. The same is true of the cells on the dorsal side where the supraoesophageal ganglia arise. Apparently many mesodermal cells also become included in the nervous system. There is little or no differentiation of tissues or structures of mesodermal origin. Mid-gut rudiments, although present, do not unite to complete the mid-gut. Hind-gut and Malpighian tubes are present, but the stomodaeum shows little development. None of the ectodermal derivatives of the anterior end, such as salivary glands or imaginal disc invaginations appear. The germ cells are present in two groups at the normal level of the gonad. There appears to be no gonad envelope. - Subsequent development is entirely abnormal. Although there is differentiation in the hypertrophying nervous system, the distribution of ganglia and tracts is irregular. Part of the hind-gut becomes well differentiated. The proventricular region of the mid-gut is recognizable. Cellular breakdown is not apparent until some hours after the time of hatching of normal larvae. Mitosis is found after this time. - The all-over effect is the development of ectodermal organs and tissues, and the failure of the others. The hypertrophy of the nervous system is at the expense of the hypoderm.

Spurway, H. and P.A.R. Street. The sex-linkage of withered in Drosophila subobscura. Withered was found in the inbred F_2 from a wild fly, where it segregated in 4 out of 8 cultures examined. The phenotype is limited to the ♀, where the penetrance is between 50-60% after outcrossing, but can be raised by selection to 100%. - Males from such a selected stock were outcrossed to a + stock. Their sons were outcrossed again, and their progeny in turn inbred in pairs. Twenty-one cultures, none containing less than 30♀♀ were examined and no wi flies observed. If the mutant were autosomal it would be expected to segregate in a 1/4 of the cultures and the probability of observing the result obtained is $(3/4)^{21}$ or 0.0024. Therefore wi is assumed to be due to a mutation on the X-chromosome. - Occasional withered males have been observed. Either their phenotype is not inherited by their sons, or it is due to an independent semi-dominant autosomal mutation.

Sutton, E. Cytological analysis of In(1)A 99b. In(1)A 99b (Stone), obtained from Texas, has an intercalary reinversion (induced by irradiation) within the limits of the original inversion. Cytological analysis of the salivary chromosomes shows the primary breaks between 1E3.4 and 2A1.2 at the left end and between 19D and E at the right end. The secondary breaks of the reinverted segment lie between 6D1.2 and E1.2 and between 19A3.4 and C1.2. The new arrangement within the chromosome is as follows: Tip to 1E3.4-19D to 19C-6E1.2 to 19A3.4-6D1.2 to 2A1.2-19E1.2 to chromocenter.

Waletzky, E. (See page 72)

Technical Notes

Blanc, Richard. Accurate timing of prepupae and pupae. It has been frequently observed by investigators of temperature-effective periods (TEP) that the TEP of a population is composed of the differing TEPs of the individuals therein (cf G. P. Child, 1935, Gen.20:127-155). This means that flies of the same chronological age may actually be in different developmental stages. In fact, even with conditions as homogeneous as possible, larvae from eggs laid in the same 2-3 hour period may pupate as much as a day apart. - The difficulty entailed by such a developmental spread may be obviated by selecting the nearest developmental landmark to the putative TEP and dividing the population into more homogeneous groups on this basis. For the study of TEPs in early pupal life, puparium formation and pupation may be chosen as characteristic stages in developmental physiology. The initiation of the prepupal period is indicated by eversion of the spiracles, cessation of motion, and loss of the definitely segmented appearance of the larvae. Young prepupae may be obtained by periodic collections and may be identified in many stocks by the color of the cuticle which is white at first and is still a very light brown at the end of the first hour. - At 25° C, pupation normally occurs at approximately 12 hours after puparium formation, although a considerable variation in time may be found. The beginning of the pupal period is indicated by a number of features, particularly by the disappearance of the large longitudinal tracheae. The writer prefers to use this developmental event as a point of reference of two reasons. First, there is evidence of a considerable individual difference in length of time of the prepupal stage, which may differ from culture to culture by more than an hour and even shows a noticeable spread within the same culture. Secondly, cultures may be collected en masse in a Petrie dish and be quickly and surely examined at periodic intervals for disappearance of the tracheae.

Brody, George. Making eggs visible on medium used for egg collections.

Several methods for making eggs more visible on the surface of the collecting medium in order to facilitate counting and removal of eggs were tried. A transparent medium (Agar 1.5gr., Crystal Clear Karo 15cc., Tap Water 100cc.), the surface of which had been inoculated by means of a camel's hair brush with a fermented molasses suspension of yeast, was used in conjunction with black trays. This had the effect of providing a black background for the white eggs. This method gave high yields only for the first few hours of egg collection. The visibility of the eggs on the surface of the medium was remarkably good. A further advantage was the smooth flat surface of this medium. For longer egg collection periods it was necessary to use cornmeal-molasses-agar-yeast medium. Charcoal added to the medium was not satisfactory; instead vital dyes added in small quantities just before pouring were used to color the medium. Those tried were Neutral Red, Methylene Blue, and Trypan Blue, of which the last was found to be most effective. There was no apparent detrimental effect introduced by these dyes. If the eggs are left on the medium for more than six to eight hours, the chorion becomes colored. This is an advantage in dechorionation of eggs since the egg itself remains white, only the chorion being colored, so that if any of the chorion remains on the egg, it is visible. Where eggs must be left on the medium, the use of a dark molasses in greater proportion, and cornmeal in lesser proportion, will provide a sufficiently dark background.

Brody, George. The use of paper boxes for collecting eggs.

Paper boxes are made in the same way as embedding boxes by folding around a 1x1x3 cm. block. One end of the box is made longer to facilitate removal from the shell vials in which they are used. The boxes are dipped in a high melting point paraffin (crude grade serves equally well) and immediately immersed in cold water. In this way several layers of wax may be added. Very little time is required for making these boxes. Eggs may be left to develop in the boxes. Since they cost almost nothing to make, they may be thrown away after using once, and thus cleanliness is ensured for every batch of eggs.

Green, M. and E.M. Slider. A method for mounting the female reproductive organs of Drosophila.

The female is placed in a drop of Ringer's or physiological salt solution on a slide and the genitalia dissected with a pair of fine needles. After the entire tract is removed from the abdomen of the female, blot the excess salt solution. Place one or two drops of 70% alcohol on the genitalia on the slide. This causes a coagulation of the organs. Occasionally the ovaries clump together; if so, they may be carefully separated with the dissecting needles. After 15-20 seconds, add a drop of 85% alcohol to the ovaries, followed by 95%, and then absolute. At this stage, carefully add a drop of colloidin (in absolute alcohol and ether) to the slide so that it runs under the genitalia. Then immerse the slide in 70% alcohol. This coagulates the colloidin and thus fixes the genitalia to the slide. After about 10 minutes in the 70% alcohol, the slide is transferred to dilute eosin or "fast green" (in 95%) for 2-5 seconds. This stains the transparent ducts of the tract. (If a water stain is to be used, the slide should be run down to water from the 70%). Then pass the slide first into carbol-xylol for 30-60 minutes, then into xylol for 30 minutes, and mount in balsam or diaphane. The preparations should never be placed in absolute alcohol after the colloidin has been added as this would dissolve the colloidin. This method enables one to make permanent preparations in a minimum time of 2 hours. The method is applicable for making preparations of the male reproductive tract although we have not made many preparations.

Kodani, M. Modified smear method for salivary chromosome preparation

The lack of proper staining capacity and the inability to render chromosomes flexible enough so they will spread out on crushing are the main difficulties with poor aceto-carmine stains. The main source of such defects is often a loss of fixing power of acetic acid when it has been boiled with carmine powder. Fixation of the chromosomes with acetic acid prior to the application of stain has been found to remove the defects. The best way is to dissect salivary glands in Ringer's solution containing a small amount of acetic acid (100 cc of Ringer's solution + 4-5 drops of glacial acetic acid). An additional advantage of dissecting the glands in the aceto-Ringer mixture is that the fat attached to the glands becomes fragile and hence very easily removed. - After the fat is removed, the glands are immediately transferred to the stain. From five to fifteen minutes of staining usually suffices; the exact time of staining has to be determined for each new batch of stain. After the glands have been stained sufficiently, they are transferred to a slide; the stain is then properly removed and a clean 45-50% acetic acid solution is flooded on the glands. All undesirable particles which may be present in the stain will be thus removed, and the glands are ready to be crushed. - In preparing permanent slides, an additional staining may be done in order to improve the smears. After dehydration in an alcohol chamber over night, the cover-glasses are taken off and both the slides and cover-glasses are placed in absolute alcohol containing a small amount of fast green stain (50 cc of absolute alcohol + 3 drops of 0.5% fast green in 100% alcohol). The slides and cover-slips are left in the solution for about one minute and then mounted in euparal. The smears thus treated show the cross-bands stained a bluish-red color while the material around the chromosomes has a greenish tinge. By means of this additional staining the small bands which are stained only faintly with the carmine are made darker and more visible.

McQuarrie, Agnes. Raising the larvae.

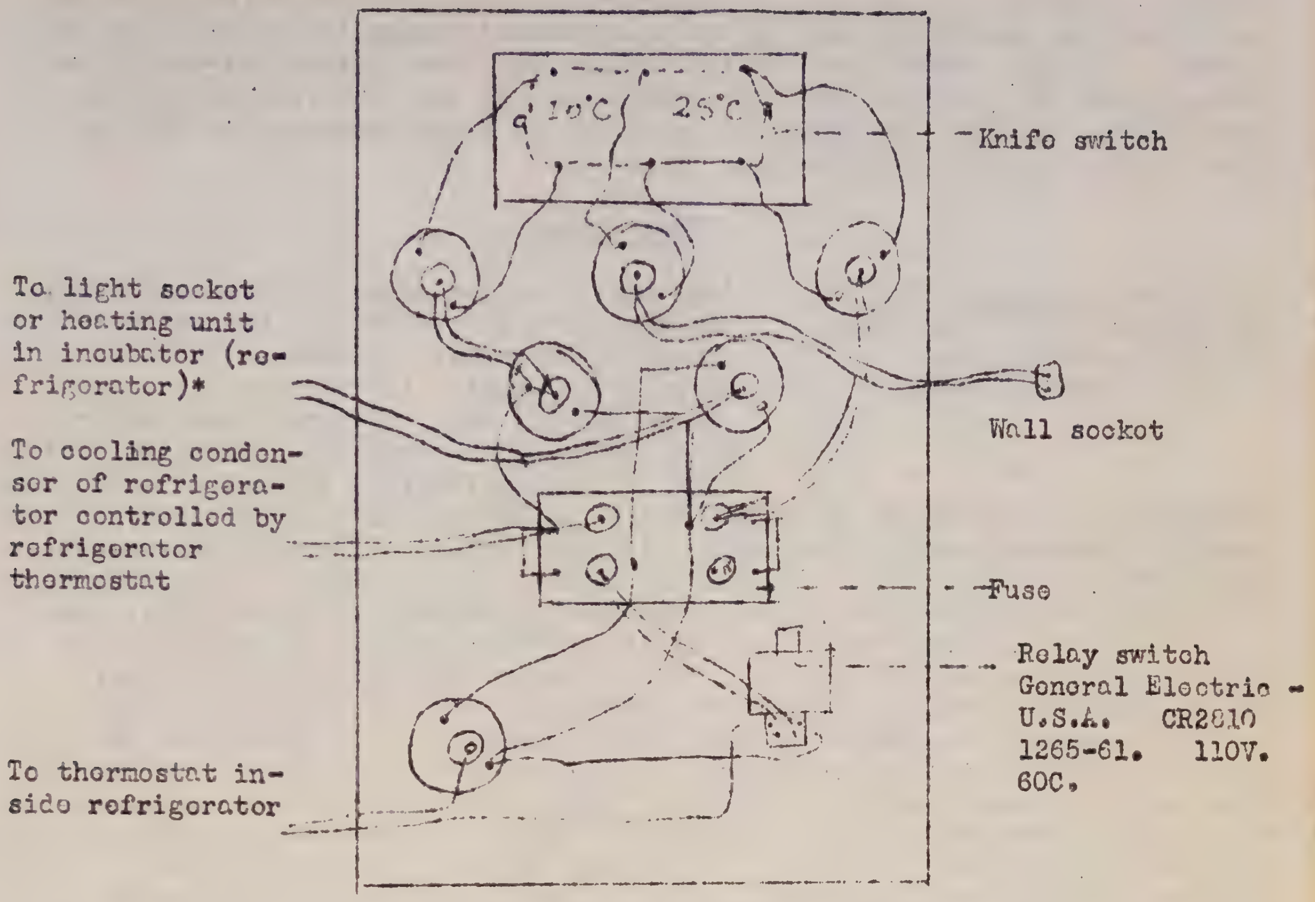
The culture that has been used in this study and which has proved to be satisfactory was the cornmeal-molasses-agar media (see Sturtevant in "Culture Methods for Invertebrate Animals", pp. 437-445). Powdered brewers yeast was added to the media and also sprinkled upon the moist paper toweling. An additional supply of yeast is added to the culture when the larvae are about half grown. - To secure the largest larvae, the culture medium must not be allowed to get too old. - The best results in larvae production were found when only a few flies, 8 to 12 adults, were released in a jar. - The bottles are set in the cold room (12-15° C.) where early growth is started. The culture bottles were left in the cold room for two weeks, or until the larvae had begun to work their way out of the medium and to the sides of the jar. - The bottles were then transferred to the ice chamber of the ice box, and it is here that the larvae undergo the remainder of their growth, usually about 5 to 10 days. Care must be taken that the cotton plugs do not touch the ice, as water will be absorbed and will inundate the medium and drown the flies. - The work was done in an old-fashioned ice box, because we had no electric refrigerator. I believe much better results could be obtained by raising the flies in a refrigerator where the temperature could be kept constant. The temperature in the ice chamber was kept as close to 5° C. as possible.

Palmer, Louise. A spoon for Drosophila egg collecting.

Pyralin, a product of the Dupont Co., has proved a very effective substance for making spoons used in Drosophila egg collecting. The grade used is about 3 mm. thick and is practically transparent. It may be bought in large sheets. Spoons are cut from the material with scissors in any desired shape to fit the culture bottle or containers for laying females. The spoons used in this laboratory are cut with handles which extend out past the cotton stoppers of the bottles or vials. Agar may be added to the spoons with a bulb pipette or funnel with rubber tube and clamp. If carefully done the mass adheres to the smooth surface and does not run over the edges. - This material has the advantages (1) that it transmits light and may be placed directly on the stage of the microscope for counts or observation, (2) that the spoons containing eggs may be dipped in water, alcohol, or other sterilizing agent, (3) that the eggs may be easily counted and divided into portions by cutting the agar and lifting out the desired number with a scalpel or section lifter, (4) that the food does not stick to the spoons but does adhere sufficiently for average manipulation, and (5) although the spoons may not be boiled they are easily ste ilized in 70% alcohol.

Quisenberry, J. H. and James E. Greer. Conversion of refrigerator to Drosophila incubator.

In addition to our regular cool-room, we use in another room an auxiliary incubator designed by Dr. E. P. Humbert. Such a unit may be quite useful to schools which wish to keep stocks through the summer for use during the regular school session without the expense of operating a large cool-room, for small laboratories, or, as in our case, as an auxiliary unit when work is also done in another room. A large General Electric refrigerator was so wired with thermostats that it can be used as a 25° C. or 10° C. incubator. The regular controls and thermostats of the refrigerator provide the latter temperature. By simply throwing the switch and defrosting the freezing unit, the box becomes a 25° C. incubator. A simple wafer or other thermostat can be used inside of the box for the 25° C. The wiring arrangement is shown by the accompanying diagram.


10°C
25°C
Knife switch
To light socket or heating unit in incubator (refrigerator)*
Wall socket
To cooling condenser of refrigerator controlled by refrigerator thermostat
Fuse
Relay switch General Electric - U.S.A. CR2810 1265-61. 110V. 60C.
To thermostat inside refrigerator

* 40 or 60 Watt globe may be used. This does not burn when switch is on 10° C. side.

Shipman, Emmet E. N-butyl technique for dehydrating Drosophila larvae

Excellent sections of larvae may be obtained by using the n-butyl alcohol technique described by K. A. Stiles, 1934, Normal butyl technic for animal tissues with special reference to insects., Stain Tech. 9:97-100. Larvae were relaxed in hot water before being placed in the fixing agent. Gilson's Mixture (nitric, acetic, corrosive, alcohol) gives good results, but other fixitives may of course be used. For dehydration, the writer used Stiles' mixtures of n-butyl and ethyl alcohols as recommended. There is a wide time tolerance for tissues in the fluids of the n-butyl series so one can arrange a schedule of changes to suit his convenience. Larvae may be stored in pure n-butyl alcohol. The writer has obtained very good sections from larvae stored for two years in n-butyl. Infiltration with paraffin must be thorough and must be slowly done. It is best to puncture the chitinous larval skin while the larvae are still in n-butyl in order to secure good infiltration when paraffin is added. The instructions given by Stiles may then be followed during the infiltration. The writer of this note will be glad to send a more complete description of the technique upon request.

Trent, S. C. Egg Collector.

For experiments requiring the observation of large numbers of eggs the following modification of the method devised by M. Schweitzer in DIS-4 ('35) has been found an improvement. It is based upon observation that the flies prefer to lay their eggs in crevices. - After the food cake of standard cornmeal agar has hardened on the paper cap, one additional drop of the unsolidified medium is dropped upon it forming a smaller second layer at the center, with the desired cul-de-sac between. After the flies have had an opportunity to lay, the removal of this layer readily exposes the eggs in a localized area and obviates the loss of time usually spent in searching for them.

News Items

Buzzati-Traverso, A. The establishment of a "Drosophila wild stock keeping center" in Pavia, Italy.

Last April the following circular was sent, together with the first list of available stocks of Drosophila species, to all the European Drosophila Laboratories (according to DIS-11) and to several other European genetical and zoological institutions: "The increasing interest in work on the population-genetics of the genus Drosophila in Europe makes it necessary to install a stock-keeping-center, where all wild strains of Drosophila species from different places should permanently be kept at the disposal of all Drosophila geneticists and zoologists interested in Drosophila systematics. As a matter of fact, those who collect flies in nature are mainly interested in one particular species or even in a certain group of geographical strains, and are not interested in keeping other ones which they accidentally catch. The wild stock-keeping-center should provide means to avoid such waste of very valuable material, which otherwise would undoubtedly be lost. On the other side, it would provide a source of systematical information and test material for Drosophila species in Europe. Since the Institute in Pavia has under way systematical work on Drosophila species, and since it is directly connected and cooperating with the Berlin Institutes (Genetische Abteilung des Kaiser Wilhelm Instituts in Berlin-Buch, and Kaiser Wilhelm Institut fur Biologie in Berlin-Dahlem) which are also engaged in population genetics of various Drosophila species, the undersigned have agreed in establishing such stock-keeping-center in Pavia. The importance and usefulness of such a center for Drosophila studies will necessarily depend on the degree of active cooperation of European geneticists in collecting flies". Signed by: H. Bauer - A Buzzati-Traverso - G. Gottschewski - C. Jucci - N. W. Timofeeff-Ressovsky. The stock lists which appear in this issue of DIS include all the different stocks received during the summer.

Buzzati-Traverso, A. Work on the population genetics of a Drosophila species belonging to the obscura group.

Since the Fall of 1938 work has been under way on the population genetics of a so-called Drosophila obscura. Such a fly has 5 rod-shaped and 1 dot-shaped chromosomes. The exact taxonomical determination of such species cannot be made for the time being, since the whole systematics of European Drosophila needs revision. By inbreeding fertilized females caught in nature a number of mutations have been obtained. Work is going on to get marked chromosomes and to make the maps of the salivary gland chromosomes. A very high concentration of heterozygous inversions seems to be present in the geographically different lines so far examined.

Buzzati-Traverso, A. "Scientia Genetica" - A new Genetics Journal for Latin countries.

Last April there appeared the first issue of the quarterly "Scientia Genetica". In this journal, whose Director is Dr. C. Jucci and the author of this note the editor, papers of Latin authors or of non-Latin authors, but written in a Latin language, are being published. A number of articles on Drosophila have already appeared. (See current bibliography).

Frolova, S. A correction

Since 1935 I have been working with Drosophila repleta. The culture was obtained by the Institute of Experimental Biology, Moscow, from a Laboratory of one of the Institutes of the United States, America. The comparison of the flies from this culture with Drosophila hydei, sent to the Institute of Experimental Biology by Professor Kikkawa from Japan, revealed neither morphological differences nor differences in the chromosome complex. - A cross, carried out not long ago, showed a complete identity of the salivary gland chromosomes in the flies taken from these cultures. Therefore, it became apparent that a culture of Drosophila hydei was erroneously sent to us instead of Drosophila repleta. - Thus, in all my works (Nature, No.3460, vol.137, Biologicheskij Zhurnal, vol.v, No. 2, 1936; Nature, No.3579, 1938; Nature, No. 3590, 1938; Bull. de Biol. et de médecine expérimentale, vol.VI, No.2, 1938; Biologicheskij Zhurnal, vol. VII, No.4, 1938) the cytological data described for Drosophila repleta belong in reality to Drosophila hydei. - A similar misunderstanding has also taken place with a culture of Drosophila robusta sent to us from the same Laboratory of the United States, America and erroneously termed Drosophila sulcata. This error was discovered in 1937 when, from the same laboratory, we received the culture of Drosophila robusta. In my two works published in 1936 (Nature, vol. 138, No.3483, 1936; Bull. de Biol. et de médecine expérimentale, vol.II, No.2, 1936) the cytological data on Drosophila sulcata belong to Drosophila robusta.

Personal News

The following is a section taken from the letter written by Boris Ephrussi on October 31, 1939: "For the time being I am out of genetics - there is practically nothing left of my laboratory, since all my male co-workers are enlisted and the girls gone. I can only keep some of my fly stocks, but it would be impossible to send flies to anybody; maybe this should be stated in DIS, as well as the following: our main worry is now to keep in contact with what is going on in the field we were working in before the war. Difficulties in keeping up with the literature are great and will gradually increase. We all will be grateful, therefore, to the American colleagues for sending reprints still more generously than before. Thanks to all in advance ! Please send also, as before, 2 copies of DIS. I hope we will use them some day again. This should show you that in spite of the profound sadness, with which we think of all the projects we had to abandon, we remain greatly optimistic in front of the present situation".

A note received from Timofeeff-Ressovsky indicates that the work in his laboratory is progressing normally with a somewhat reduced staff.

Buzzati-Traverso is struggling to keep up with his research at intervals while on leave of absence from military duty.

B. Slizynski and Mrs. Slizynska came to Edinburgh to attend the Genetic Congress and were not able to return home. They are now working at the Institute of Animal Genetics at Edinburgh.

Additional Research Notes

Waletzky, E. Interaction of bifid with other wing mutants.

Previous work has shown the existence of a disproportionate combination effect of bifid with various Beadex allels and with scalloped. Qualitative examination indicates that bifid does not interact disproportionately with miniature or radius interruptus. Disproportionately small wings are formed in the bifid clipped and the bifid vg^{nipped} combinations. No effect of bifid on vg^{vestigial} was detectable. The embryonic development of these wings is being studied.

Waletzky, E. Manifold effects of Wrinkled.

Reduced wings and abnormal black pigment spots on the head are manifold effects of Wrinkled. The manifestation and the penetration of these two characters are correlated in + W/++ flies. The smaller and more abnormal the wing, the greater the frequency and intensity with which the pigment appears. When the wing is practically normal in size and shape, abnormal pigment is absent. These relations also hold in D +/+ W and D^3 +/+ W flies, which frequently have practically normal wings. However, the penetration of the pigment character is not increased in D +/+ W flies whose wings are reduced by the presence of miniature or dumpy. W/W flies, whose wings are very greatly reduced through the presence of vg^{vestigial}, show no pigment. Similarly the penetration of the pigment character is very low in W/W flies whose wings are greatly reduced through the presence of vg^{nipped} or Beadex^J. Further combinations are now being studied for their effect on these correlations.

Research Notes

Ahmed, I. A. A new inversion in the third chromosome of D. pseudoobscura.

By means of X-rays (4,500 r units) an version was induced in the third chro some of D. pseudoobscura, which is kn have 17 different gene arrangements found in natural populations. The new inversion was first detected genetically by the absence of single crossing-over along th or part of the third chromosome. In the salivary gland chromosome the bre points were found to be in sections 65C and 81C, according to the map of sky (1938, Genetics 23). The third chromosome in flies heterozygous for version forms a very large loop with a short handle. This is the largest hitherto observed in the 3rd chromosome of D. pseudoobscura. The distal point is at the very tip of the chromosome and probably not more than one bands are left in the original position. They may represent the telomere. genetical data suggest that the gene orange is the most proximal gene. B which is 7.7 units distant from orange, is located between the centromere proximal breakage point.

Alikhanian, S. The effect of deletion upon the manifestation of a gene altering wing shape.

A curly-like mutation has been obtained X-chromosome of D. melanogaster while X- the y v f B stock. The peculiarity of t mutation consisted in the fact, that in the presence of dl 215 the wings were bent d while in the absence of this deletion they were lifted up.

Alikhanian, S. A dominant mutation with manifold manifestation.

The y v f B males were X-rayed and then y ClB.y w females. One of the y ClB/y f from this cross was mated to a male, carrying the dl-215, covering the region of the left end I,2B. No males were obtained from this mating. The fact indicated the pr of a lethal somewhere in the sex-chromosome. Half of the y v f B/y females manifested the character of the c. i. type and the reduction of bristles of th ac type.

Barigozzi, C. X- Inversion in a mottled strain, and Notch without deficiency.

The studied strain (mottled Notch 218) i tive to high temperatures, and dies at o - Males mottled are ordinarily missing a few appearing are sterile. -Mottling and Notch are widely variable. - An inversion in X-chromosome was observed, fr mocenter to locus white. No deficiency appears in relation to Notch. (In by Rend. Ist. Lombardo Scienze e Lettere).

Born, H.J. and Zimmer, K.G. The influence of incorporated salts of heavy metals on the frequency of X-ray induced mutations.

Stadler, Medvedev and Buchmann have show the X-ray induced rate of mutations is i by the incorporation of salts of heavy m before irradiation. This effect has bee ed to be due to the increase of energy a sor tion within the tissues. The explanation seemed, however, open to doubts, as quantitative data on the amounts of heavy metal, deposited in the gonad not available. We have, therefore, made quantitative determinations of ir lead in the gonads of flies reared on iron and lead containing food, using chemical methods as well as "Dagging" with radioactive isotopes. The resu that the amounts of neither iron nor lead are sufficient to cause the incr energy absorption necessary to explain the observed increase in X-ray indu of mutation (these amounts being of the order of magnitude to allow an inc the rate of mutation by about 1%, whereas the latter is really of the orde of about 50%). These experiments, therefore, do not support the above men "physical" explanation of the effect. - The same conclusion was reached in experiments carried out in cooperation with Dr. Buchmann, where the wave-l dependence of the increase of mutation rate by incorporated iron was studied. Because of the considerable wave length dependence of the absorption of iron, the "physical" explanation would require the same for any biological effect due to absorption of radiation in iron. In fact only a small and statistically quite insignificant wave length dependence could be found. - Although both of these investigations are in good agreement in so far that they do not support the "physical" explanation, we think that the matter should be investigated further, and we are planning more detailed experiments.

Buzzati- Traverso, A. A singed melanogaster male caught in nature. On November 19, 1939 together with about 200 normal melanogaster flies, one singed male was caught near Pavia. 112 fertilized females were tested to see whether they were heterozygous for this mutation. None proved to be so. Out of one of such females a line was established, which seems to be highly mutable. More than ten mutations have been found, among which a case of reoccurence of singed. It is likely that the singed male found in nature was a mutant of a free living population highly mutable. Experiments are under way to test the rate of spontaneous mutations in this line.

Buzzati-Traverso, A. Some new Drosophila species belonging to the "obscura" group. From the study of material collected in Italy, Germany and England, it has been possible to F.P. Pomini and the writer to distinguish five different species within the "obscura-group" of Europe D. One is the already described D. subobscura, which is present in Germany and Italy. To the other four species the following names have been tentatively attributed, before deciding whether one of the described species may be homologized with D. obscura described by Fallen in 1823: D. obscuroides (2 V-shaped, 3 rods and 1 dot-shaped chromosomes), D. bilineata (4 V-shaped and 1 dot-like chromosomes) D. tristis (4 V-shaped and one dot-like chromosomes) and D. Haldanei (chromosomes not yet analyzed).

Buzzati-Traverso, A. A new species of drosophila with variable number of egg-filaments. A not yet described species of Drosophila has been caught in Pavia and Treviso (Italy). It is characterized by its shining black appearance, by the fact that (at least during the winter) it reaches sexual maturity only after two months of adult life, and by the fact that eggs are laid having 4,5,6,7 or 8 filaments. The same female lays eggs of different type. Experiments are under way to check the inheritance of such egg character. The specific name: Drosophila nitens is proposed.

Calloway, S. and Kalmus, H. Recovery time after ether narcosis by means of a physiological analysis in the species and races of Drosophila. A simple method of etherization giving consistent results, is as follows: Into a wide-necked jar a measured quantity of liquid ether (resulting in a vapour concentration of 0.2-5%) is poured and the closed jar shaken several times. Then the flies are dropped in and remain there for a measured time (½ - 40 minutes). Recovery times of individuals or batches of about a dozen flies are measured. Newly hatched flies usually recover more quickly than older ones. Females recover more quickly than males. After slight narcosis (lower ether concentration or shorter periods of etherization) melanogaster and simulans recover earlier than subobscura; in high concentration the behavior is reversed. D virilis recovers earlier than the other species at all concentrations. Some mutants recover more slowly than the corresponding normal stocks, whereas in others no significant difference appears. Analysis of the relationship between time of recovery and intensity of narcotization is still in progress.

Chino, M. and Fujii, S. Correlation between the gonial chromosomes of In the gonial metaphase plates of D. virilis virilis, both male and female

D. virilis americana with the linkage groups of D. virilis virilis.

have six pairs of chromosomes, five pairs and one pair of dots. The americana fema four pairs of chromosomes, two pairs of V's, one pair of rods, and one pair of dots. The americana male differs from the female in having one of the V's replaced by two rods. One of these two pairs of V's involves the X ch We found that one arm of this V-shaped chromosome is the fourth and the ot the X chromosome. Another V-shaped chromosome consists of two autosomes, second and the third chromosomes. The rest, rod-shaped and dot-shaped chr correspond respectively to the fifth and the sixth chromosomes.

Eloff, G. Cytology of South African species of D. busckii.

Metaphase cells from supra-oesophageal ga larvae and oogonia of imagines from cultu S. African D. busckii show the presence of 3 pairs of chromosomes, - two large V-shaped pairs of appr. equal length, and a more or less rodlike pair. As far as t of chromosomes are concerned the results agree with those of M.I. Sirotina DIS-11 p. 49). It was not possible to observe a satellite on the proximal the rod-like chromosomes, although salivary gland nuclei justify its prese - The salivary gland nuclei show 4 separate elements: 2 large chromosomes the same length, and each showing a constriction dividing one of the chrom into appr. equal halves. The latter chromosome may be readily distinguish the other by the presence of a transparent, almost nucleolar-like swelling ing no bands across the middle area, and situated at appr. one fifth the d from the free end of the arm. Of the remaining two elements, one measures half the length of the V-shaped chromosome with the said swelling. It is to represent the rod-like chromosome, i.e. the X-chromosome, of the gangli and the oogonia. Lastly, there is a very small element appr. one fifteent length of the X-chromosome. This probably represents the satellite referr above in connection with Sirotina's work.

Grant, J. Primrose and Eloff, G. The effect of the sex-linked genes yw on chronological sex-ratios in D. melanogaster.

Data obtained in connection with daily co offspring from Drosophila cultures in whi ents were left for 48 hours, pointed in t of the criss-cross generation to a high o frequency on the fifth day of counting, especially accentuated in the case of the males. In the case of the pure yw cultures the females showed a strong fifth day peak. The phenomenon which is proba associated with lowered viability, was absent in the case of wild cultures melanogaster and D. simulans. Here the highest counts were obtained on th day of counting, whereafter the decline was sharp and regular. The practi portance lies in the fact that with regard to the criss-cross generation, ratio will apparently be radically different for the first two or three da count from the fourth and fifth days of counting. An experimenter countin three or four consecutive days must of necessity obtain a higher female po than a worker counting for five or six consecutive days.

Hadorn, E. Maternal effect on lethality.

It was possible to select from the "letha larva" stock (lgl on bw/Cy) two genotypes differ clearly in the time at which the d the homozygous lgl/lgl occurs. In one lin practically all of the lgl/lgl zygotes die in an early embryonic stage; in other line most of the lgl/lgl zygotes reach the stage of full-grown larva Crosses and backcrosses between the two lines show that the time of death of lethality) is determined by the genetic constitution of the heterozygou mother and not by the genes of the dying lgl/lgl zygotes within the new ge

Neuhaus, M. On the incomplete dominance of normal allelomorphs in 3N females

In DIS-7 a triploid stock of *D. simulans* described by us. It was pointed out tha triploid females had forked-like bristl has been found since those females contai normal allelomorph of sn in one of the attached X-chromosomes, while the o attached X and the free X-chromosome carried the mutant allelomorph of sn. females having one mutant sn gene possess normal bristles. It is therefore that the normal allelomorph of sn show incomplete dominance only over two r allelomorphs of sn. - The normal allelomorph of the w gene shows as well ir dominance over two mutant allelomorphs of w triploid females of the follo type $w^{+}/w/w$ have a plu like eye color. - The normal allelomorph of yellow complete dominance over two mutant allelomorphs of y. - It is interesting that in D. melanogaster triploid females of the genotype yy_{+} differ from of the constitution y^{+}/y the former being lighter, which is especially well ceptible in tergites. Two normal allelomorphs of yellow have been studied *melanogster*, one in the sc^{8} line, while the other in the Florida stock. Bo incomplete dominance over two mutations of yellow. Unfortunately in D. si in spite of the considerable material studied, we failed to detect 3-X-chro females. In D. melanogaster such females of the constitution $\underline{y}\ y^{+}$ 2A poss darker tergites than do the triploid females of the constitution $\underline{y}\ y^{+}$ 3A.

Oliver, C. P. Crossing over between a pair of alleles in D. melanogaster.

In fertility tests on lz^{s} and lz^{g}, a smal frequency of crossing over occured betwee alleles in the compound lz^{g}/lz^{s}. The eye lz^{g}/lz^{s} females are similar to the eyes of zygous lz^{g} females; in lz^{g}/lz, the eyes are blended. Each compound and eac zygous females lacks spermatheca and parovaria. From the compound lz^{g}/lz^{s}, imately 0.14% of the offspring have -type eyes and normal genital tracts. unexpected reversion types occur only in offspring from the compound lz^{g}/l are associated with crossing over. The complementary type, which should ha and lz^{s} on the same chromosome, has not yet been recovered, either due to t failure of that type to live or due to the inability to differentiate the t from the other genotypes. Whether unequal crossing over is involved, or wh Bridges' repeat hypothesis is involved in being investigated.

Pilkington, R. W. Notes on the development of some facet mutants.

The roughness of the eye surface in fac ants appears to be due partly to an und of the surface (Gl, spl) and partly to i ularity in the shape of the facets causing absence of the three-directional of the uniform hexagonal facets of the wild type. In ru the facets are re hexagonal but the rows are not straight. In Gl the facets vary greatly in the number of the sides to each polygon varies from 4 to 8, and there are a of small facets of irregular shape. In ro and spl the polygons have 5 to 7 - It is possible to see the retinula cells below each facet in the living p an oil immersion lens as soon as the eye is pigmented. A relation between bor of sides to the facet and the number of retinula cells can thus be made In Gl and spl the number of retinulae is equal to the number of sides to th gon; and the wild type appears always to have 6 retinulae. In ru the relat does not hold good there being 6 to 8 retinulae to each hexagonal facet. F examination is in progress.

Pilkington, R. W. The development of forked bristles

The thoracic bristles usually show a defi symmetry of the corresponding bristle on sides of the body, but this symmetry is r universal. The small hairs of the wing are often bent, but show no forking bristles and hairs which have a sharp bend, the pigmentation may be comple the socket up to the bend, while the portion beyond the bend remains color may take more than 24 hours for the pigment to turn a sharp bend, but once got round, the darkening will spread up to the tip of the bristle in two o hours. The same effect was noticed in wild type pupae examined alive under a cover glass; the glass apparently pressed on some of the bristles, causing kinks in them. If the glass was removed, the bristles would straighten out, and the pigmentation would then progress within a few hours to the tips. (These observations were made by means of the technique described elsewhere in this issue.)

Pontecorvo, G. Further investigations on the sc Y^L chromosome. Cytological data by Koller and Slizynski and genetical by Pontecorvo, Lamy and Muller show that the sc Y^L chromosome (Crew and Lamy, 1940, J. Gen., 39: 273-283) is V-shaped, one of its arms being the Y^L, and the other having a length equal to, or a little greater than, that of a normal X. - The bb^+ gene-perhaps duplicated- is present only in this latter long arm. For this long arm the following constitution is provisionally suggested, proceeding from the spindle fibre attachment: (1) the proximal region of the Y^S up to bb^+ locus (Neuhaus, 1939), including the main block of Y^S; (2) block A, (and possibly bb^+ and bk B) derived from the X chromosome; (3) the terminal portion of the sc^{S1} X-chromosome from its left break - immediately to the right of sc - to the tip. As foreseen in the previous paper (Crew and Lamy, 1940) the sc Y^L has already proved to be very useful in investigations in which it was necessary to observe the distribution of a Y chromosome. Males containing the sc Y^L and an XY^S give some 0.04% exceptions by primary non-disjunction. An X-chromosome in which the region from sc to the tip is duplicated has been obtained by crossing over between attached Xs and the sc Y^L; this process produces a V-shaped X-chromosome the right arm of which is the long arm (containing sc) of the sc Y^L described above. - Some confusion may arise from the terminology in this case since the long arm of the normal Y (Y^L) here forms the shorter arm of the sc Y^L chromosome, but it seems best to adhere to the original method of denoting Y^L.

Pontecorvo, G. Non-disjunction of the autosomes. By a method which will be described in detail, it has been possible to measure non-disjunction (as well as X-ray induced loss) of the autosomes. In males heterozygous for Cy L^4 and DCXF primary non-disjunction of the second chromosome occurred with a frequency of some 0.08 ±0.04.

Pontecorvo, G. Rate of egg-laying at low temperature. Observations connected with other investigations in progress showed 13°C to be the lowest temperature at which the rate of egg laying is still of practical use. y; bw; e; ey females, not more than 24 hours old, were placed with males for 3 days at 24°C. They were then separated from the males, put at various low temperatures (including series at ca. 8° and 10°), and transferred to new food every day or two. At 13°C they reached a maximum rate of deposition of 7 eggs per fly per day on the 7th day. This rate was sustained with small variations up to the 12th day (16th from hatching), when a sharp decline began. No doubt wild type flies would lay more eggs and have a more sustained egg production.

Shimakura, Kyojiro. The use of a non-fermentative yeast in Drosophila culture. *Torula rosea*, a non-fermentative yeast whose colony is pink in color, has been found usable in Drosophila culture. It never causes the displacement (slipping out of position or falling down when the culture bottle is inverted at etherization) of the food cake, even if ordinary milk bottles or cylindrical vials are used as culture vessels. The propagation power of this yeast does not decline so quickly as that of Saccharomyces cerevisiae, thus allowing more flies to be raised in one bottle and particular stock cultures to remain safe for a longer period of time. Unfortunately, however, *T. rosea* is apt to be more easily overwhelmed by some fungi than *S. cerevisiae* and it is desirable, therefore, that the flies to be put in the bottles of the former yeast are mycologically more or less cleanbodied. Detailed accounts in these connections will shortly appear in Japanese Journal of Genetics.

Singh, R. B. Prolongation of larval life in Drosophila melanogaster.

In connection with another investigation secondary experiment was set up to lengt period between the laying of eggs and emergence of adult flies (D. melanogaster) from 10 27-30 days without changing the temperature (23°C). The following is th of technique and results. - Fresh males and females were mated together ally in vials. After 72 hours the females were separated from the male in pill-boxes which had netting on one side and were closed on the other. The pill-boxes were placed on ordinary food without yeast with their netting down on the food, so that the flies might take food and lay eggs through netting. After the flies had laid a considerable number of eggs, they w re-moved, with the boxes, and the eggs were transferred with the food to a Twelve hours after the hatching of the eggs, the larvae were transferred ordinary food to syrup food. Every sixth day they were transferred to n food till the 17th day, when they were transferred back to ordinary food hours. Finally they were taken to syrup food again, where they pupated emerged from the pupa case 27 to 30 days after the laying of the eggs. - contrast with this, if the eggs were laid on yeasted food and kept there whole larval period, they would reach the imago stage some 10 days after ing of the eggs. - This kind of starvation, which results in delay in the ity of the flies, has a great influence on their growth. The starved fl much smaller in size and weight than the normal ones. Their eye colour i lighter in the beginning than that of freshly hatched flies that had a n developmental period. 47 flies of the starved group and the same number control were weighed one day after emergence and their weights were .021 and .057 gms. respectively.

Singh, R. B. Yellow in scute-8 chromosome.

A yellow has arisen by spontaneous mutation in 8 chromosome. This yellow, designated as y^{Si}, phenotypically like the typical (extreme) yello the stock thus provided (see Edinburgh stock list) is so far as we know only scute-8 stock available containing a typical yellow, the others with yellow alleles having dark bristles. In this stock (y^{Si} sc^8 B w^a) the cr over seems to be of the usual frequency for the scute-8 chromosome.

Stubbe, A. and Vogt, M. Comparison of the wild types of D. melanogaster, funebris, and pseudoobscura, as determined by transplantation of eye-anlagen.

Up to date relatively little is known differences in the developmental physi of the different species of Drosophila Since the introduction of the method o transplantation in Drosophila by Beadl Ephrussi, a new method of approach to investigations is available. Gottschewski and Tan were the first to revea transplantation of eye-anlagen quantitative differences in the supply of v and cn$^+$ - substance between the wild types of D. pseudoobscura and D. mela gaster. In this connection a comparative study of the wild types of diffe other species of D. might be of some interest. - In a first series of expe we were able to show that the wild type of D. funebris differs from the wi of D. melanogaster by a reduced amount of cn$^+$ - substance in the body lym At the same time, the wild type of D. funebris shows a high production of v substance, and differs thus from the behavior of the wild type of D. pseudoobscura as described by Gottschewski and Tan.

Stubbe, A. and Vogt, M. The homology of some eye color genes of D. funebris and D. melanogaster, determined by transplantation

The homology between the genes v and cn of I funebris with the corresponding genes of D. melanogaster was established. The gene st o D. funebris behaves like the genes of the st group of D. melanogaster.

On account of the differences in the production of the v^+ - and cn^+ - substance in the wild types of both species, it is not possible to decide whether st(f) is homologous to st(m) or to cd(n).

Timofeeff-Ressovsky, N. W. and Zimmer, K.G. Production of mutations by radiations with very high spatial density of ionization.

It has been shown by Timofeeff-Ressovsky and Zimmer and by Fricke and Demerec that there is no influence of ionization density in the region from gamma-rays of Radium to 10kV-X-rays. From this and from the dosage-mutation-curve it could be deduced that the occurrance of one ionization within a certain volume (target volume) is sufficient for the production of a mutation (Timofeeff-Ressovsky, Zimmer and Delbruck). It is evident that any radiation, producting ionization of such a spatial density that in the average more than one ionization (if any) occurs within one target volume, should be less efficient in the production of mutations. We have failed to demonstrate this effect using very soft X-rays and alpha-rays from radon, but were sucessful in using fast neutrons from a high tension neutron generator. Actually we found a loss of "efficiency" in producing mutations of 30 - 40% as compared with X-rays. This result depends of course, greatly on a reliable method of comparative dosimetry for X-rays and fast neutrons. The ionization-chamber-method used in our experiments has recently been checked by Gray, and found to be sufficiently accurate. Using Gray's values instead of ours the loss in efficiency would still be increased. - Depth dose (absorption) measurements with neutrons, carried out by one of us (Z.), make sure, that the loss of efficiency cannot be due to in homologeneous distribution of radiation within the flies' bodies. - Our results, therefore, must be regarded as further support of our view on the process of induction of gene mutations.

Valadares, Maria. Cytological evidence of spontaneous somatic chromosome-rearrangements in D. melanogaster.

In the same salivary gland of larvae coming from the previously described highly mutable stock of D. melanogaster (Valadares 1938), I have observed cells with normal chromosome and cells with a number of chromosome rearrangements. Inversions, deficiencies, translocations and duplications occur with a relatively high frequency in this stock. Among such chromosome-rearrangements the following have been identified: In(2L)391; In(3R)40a; Df(2R)40a; T(1;2R)40b. Since such spontaneous rearrangements occur in a stock whose mutations rate seems to be extraordinarily high, it is likely that this two phenomena are interdependent. A series of experiments is under way to check exactly the gene- and chromosome-mutation rate of this stock.

Varshaver, N. The effect of duplications of the left end of the X-chromosome on sex in males of D. simulans.

A study of the effect of 13 X-chromosome duplications on sex in males of D. simulans was undertaken. All duplications produce a visible effect upon the morphology of testes, causing the appearance of aberrant forms. The percentage of these aberrant males among males carrying duplications varies from 3.0% in dl 27 up to 39% in dl 24. The examination of external genitalia of such males revealed that three duplications beside producing the mentioned effect on testes, cause the appearance of abnormal genitalia. The percentage of such aberrant males varies from 18% in dl 27 to 66% in dl 24. The comparison of the morphology of aberrant forms arising under the influence of left end duplications with the morphology of triploid intersexes reveals beside some pecularities similar features in changes of gonads and external genitalia. Aberrant males carrying duplications were therefore designed as diploid intersexes. - The cytological study of duplications has shown that the shortest duplication contained the region 1B3.4, the longest-the regions I, 2, 3A3 4 of the Bridges map. - The comparison of the effect of duplications on sex with their cytological length has enabled us to design regions of the left end responsible for this effect. Duplications for 1A-1B3.4, 1E3.4-2A3 and 2-3A3.4 regions produce a considerable influence upon the morphology of the gonads, while duplication for 1B3.4 - 1E3.4 a 2B regions are neutral in this respect. At the same time duplications for the region 2B exercises a great influence upon the morphology of genitalia. So does as well the region 2-3A3.4. - The results of the cytol and genetical study, as well as the study of the correlation between the c in gonads and external genitalia, which is practically absent, has enabled assume that the observed changes of testes and external genitalia are caus the influence of different genes. Since the influence of duplications off most fundamental primary sex characters, producing changes which partly ar to intersexual changes at triploid intersexes, this phenomenon is consider due to the effect of sex genes of the left end, tending the normal develop the male to female direction. The data obtained support Bridges conceptic cerning sex determination in Drosophila. In fact it is obvious that we ar here with a number of genes, all of them influencing sex, without producin reversal as should do the main sex gene. Our data have enabled us to say genes can differ not only in respect of their strength, but as well in res the character of their action, which does not necessarily affect all sex c but can be restricted to some of them only. - The comparison of the effect simulans duplications on sex with the effect of D. melanogaster duplicatic same cytological length, has revealed considerable differences. While, as mentioned above, the region 2B in D. simulans produce a strong effect upon morphology of external genitalia, in D. melanogaster it is neutral in this

Waddington, C. H. Development of wings. The German students of insect wing development (pupils) divide each instar into four phases; (1) (2) withdrawal and cell-division, (3) stretching folding, (4) chitin formation. Following this, the propupal and pupal ins Drosophila may be divided as follows:

Stage	Age (25°C)	Events in the wing
PP 1	0-4 hrs.	Wing everted, bends into transverse plane.
PP 2	4-6 hrs.	Wing flattens, propupal venation easily visible.
PP 3	6-9 hrs.	Wing expands and becomes thin, but not much fold rotates into long axis of body.
PP 4	9-12 hrs.	Wing begins to inflate, venation disappears.
P 1	12-19 hrs.	Wing a thin hollow sac.
P 2	19-45 hrs.	Wing retracts, definitive veins formed.
P2a	19-20 hrs.	Marginal thickened ridge.
P2b	20-21 hrs.	Veins distally and proximally, vesicle in centre
P2c	21-28 hrs.	Full venation appears.
P2d	28-45 hrs.	Veins become narrower, epithelium more dense.
P 3	45-60?	Wing folds.
P 4	60?-emergence	Chitin formed.

Some further data on the development of mutants, subsequent to that publis Nat. Acad. Sci. 25, 299, has been obtained. Abnormal (dummy-type or Blade Contraction in P2 is found in all wings whose original shape is abnormal t of scalloping. This angle between L2 and proximal part of L5 is about 29° smaller in narrow types (Ly 25-26°, Bx^C 22°) larger in squarer types (Xa 32 The space between the pupal sheath and P2d wing of vestigial is quantitati accounted for by this, ~~is one remembers that~~ - Shifted-2 is a deformation of longitudinal veins, since L2 and L5 are pinched together as L3 and L4. It is visible in PP2, and probably happens before eversion of Dachs, approximated and four-jointed are general deformations in opposite veins being splayed, particularly at ends; angle between tips of L2 and L5 against 37 in wild. The shifting of C1 distally and C2 proximally is prob lated to this splaying. C2 also shifts proximally in dumpy, and this is r ly due to deformation, but is a real movement relative to the epithelium. suggests that the position of C2 is not determined until P2, and this is s by the fact that C2 may be shifted proximally in dp; vc flies in which L5 short (vc does not act visibly until P2,). - General deformations (broad, lanceolate-2, narrow) are visible in PP2 or PP3. Probably due to affects division in PP2. Shape of cells (estimated from hair distribution in adul

if one remembers that basal part of wing (mostly vein material) normally contracts more than distal part same as wild, thus effect is on rates of division in different directions. In Wrinkled the narrowing of the base to form the wing insertion starts prematurely (in propupal) and progresses too far. This probably interferes with the flow of body fluid in the unfolding after emergence (cf. interaction with bifid).

Waddington, C. H. Notes on development of eye and body colours. Pryor (Proc. Roy. Soc. B 1940, 128, 378) has shown that darkening of chitin in many insects is due to tanning by a dihyrdoxyphenol. The ferric chloride colour test for such substance (green, going red with alkali) can be obtained with pupal eyes which are just coloured brown. The eye is immersed in strong alcoholic ferric chloride for some minutes, then removed to water, when the green colour appears in the eye (with luck !). It has never been obtained with eyes lacking the brown pigment (vermilion, scarlet). - Pupa cases can be darkened by tanning with benzquinone, and so can body cuticle if treated at a time when it is ready to darken naturally, e.g. just emerged flies. Pupa cases of ebony are considerably paler than those of wild; in benzquinone they rapidly darken to wild colour, and subsequently both they and wild continue to grow darker more slowly. Body cuticle of ready-to-hatch yellow darkens through wild colour to something darker than adult wild in 24 hours; wild cuticle also becomes darker than adult wild, but ebony does not get as dark as adult ebony (difficulty of diffusion ?). It seems that the fly only produces a limited amount of tanning agent, insufficient to saturate the protein present. The action of ebony is to prevent this being liberated early enough to darken the pupa case fully, so that more is left to tan the cuticle of the adult. This accounts for its behavior as an antimorph.

Waddington, C. H. and Loos, A. D. Determination of the pupal wing. Work is proceeding on the effects of injuring the wing with a needle. In operations made in the white propupa or later, pieces may be removed from the margin with no correlated regulation of the venation; thus the longitudinal veins are at least partially determined at this time. No cases have been found in which upper and lower vein-surfaces were disassociated, so that veins are probably determined on one surface only. No holes are caused by operations earlier than 6-7 hours, but the venation is sometimes extensively messed up, probably owing to wounding and repair of the central regions of the wing. General deformations of the whole wing are found. Some of these are dumpy-like, and due to abnormal contraction but others are more like broad and may be due to abnormal cell-division and/or expansion in PP2 and 3 but are not yet analyzed. Gaps in L3 (like tilt) occur frequently; the condition of the nerve and tracheole in these is not yet certain. Later operations which upset contraction give bloated-like (or less extreme) wings. The position of the posterior cross vein can be shifted by operations made as late as 9 hours (later not yet tried.).

Wenstrup, Edward J. Crossing-over in the yellow white region of the sex chromosome in D. melanogaster. In the course of experiments on the effect of decreased pressure on crossing-over in D. melanogaster it was noticed that there was an increase in crossing-over rate between yellow and white as the experiment progressed. This held true for both the control and the experiment and was contrary to what Bridges, Plough and others found for the sex-chromosomes and autosomes. To test this further a second mating was made and the first generation untreated females that hatched out during a 24 hour period were used, 60 females in all. The first generation females were changed daily for 40 days. Grouping the results into ten day periods follows.

1st ten day period 5632 flies with 74 cross-overs. .0131
2nd ten day period 5345 flies with 186 cross-overs. .0310
3rd ten day period 5356 flies with 269 cross-overs. .0502
4th ten day period 3968 flies with 178 cross-overs. .0448

Comparing the first ten day period with the second, third and fourth the probability of error is 6.5, 10.9, and 8.6 respectively. - A test of the remainder of the chromosome involving yellow, vermillion and forked showed no increase in over with age of the females, confirming the results of others. 21°C.

Technical Notes

Fink, H. K. To prevent flies sticking in medium

Despite the improvement in D. mediums by addition of agar to jellify the mixtures od, there remains as ever the danger of experimental flies getting stuck, especially young virgin females (with moist Some workers, of course, put their etherized flies in paper cones before place them in bottles, others spread the paper toweling in such a way as a shelf over the bottom of the bottle, while some slow-breeding species a in vials for a day before transferance to bottles (to lessen chances of n Whatever system used, flies still may get stuck by getting beneath the p and dipping their wings accidently into the food. As a special precautio some workers here at Caltech and elsewhere have sprinkled fine sawdust ov medium, but this has one disadvantage in that it tends to drop out along the flies when they are etherized. At practically no expense, I have tri found most successful the use of easily-procurable wheat germ, sprinkled medium in the same manner as the saw dust and any excess immediately shak into another bottle. Each particle of the cereal that reaches the agar f bond with it, absorbs moisture from it, and will not drop out later to me the flies to be observed. The expense is small, considering the very min amount necessary in each bottle. In addition, the cereal probably afford of the essential vitamin B. in the fly's diet. To determine whether the germ is actually eaten, melanogaster flies have been grown on the (dry, u cereal alone (moistened slightly with distilled water). The bottles were moldex, so some became moldy, but in mold-free bottles, larvae grew fat o food (or possibly microorganisms growing on its surface). Pupae appeared seventh or eithth day and emergence was normal. Wheat germ is not recomm as a medium for fruit flies, but is is interesting to find that they can and apparently thrive on it alone.

Haskell, G. M. L. The useof chondrus to replace agar in food preparation.

Chondrus (Irish Moss, Carrageen) is a sea which is dried and sold as yellow, horny It may be used as a substitute for agar i preparation of cornmeal-molasses medium. Chondrus must first be ground to powder as in the unground state it will mix properly with the cornmeal on heating. This grinding is done adequat an ordinary meat grinder. The agar should be replaced by twice its weigh Chondrus, but otherwise the preparation is unaltered. As compared with A Chondrus has two serious disadvantages, viz. (a) it must be ground before and (b) the hot chondrus food is not so easy to pour as agar food. Flies however, breed well on chrondrus medium and so, in the event of difficult obtaining agar, chrondrus should prove to be a useful substitute.

Koller, P. C. Technique for staining mitotic chromosomes for Drosophila.

During the past two years a technique has worked out which appears to be excellent study of chromosomes in Drosophila. The of larvae are dissected and left for 4 to 5 minutes in Carnoy or in a modified form of Carnoy (12 cc. absolute alcoho 6 cc. chloroform, and 1 cc. acetic acid glacial). The superfluous tissue separated from the ganglia, which are then transferred into aceto-carmine by evaporation. After staining, the ganglia are transferred on to a sli a drop of gresh aceto-carmine, covered with a cover-slip, and gently pres the superfluous stain being dried up with blotting paper, and the cover-s is then sealed with wax or other sealing material. The slides can either studied immediately or can be left for a day, when the chromosomes will b stained and will stand out more clearly. The chromosomes, if treated in this way, do not swell and do not contract, but remain thin and slendor, and the coiling of sister chromatids can be very clearly seen. - The same method was used for chromosomes in the spermatogonial cells of D. miranda, and was also found that chromosome association in theprimary spermatocytes of D. miranda could be stained very well in this way. Experiments with the chromosomes of salivary glands have shown that if they are pretreated with Carnoy, banding of the chromosomes shows up more clearly.

MacKnight, R. H. and Herschel Roman A culture medium without agar.

A decreasing supply of agar in certain markets, together with increasing cost, recommend the use of some other substance in the preparation of food for Drosophila. Various modifications of the formula using cornmeal have been tried, but in general the food crumbles and is shaken from the bottle with the flies. This objection may be largely overcome by the use of rolled oats instead of agar in the formula. Since cornmeal and rolled oats differ according to the source from which they are obtained, the following proportions may require some alterations: -

Cornmeal	- 20 grams
Sugar	- 15 grams
Rolled oats	- 5 grams
Water	- 60 grams

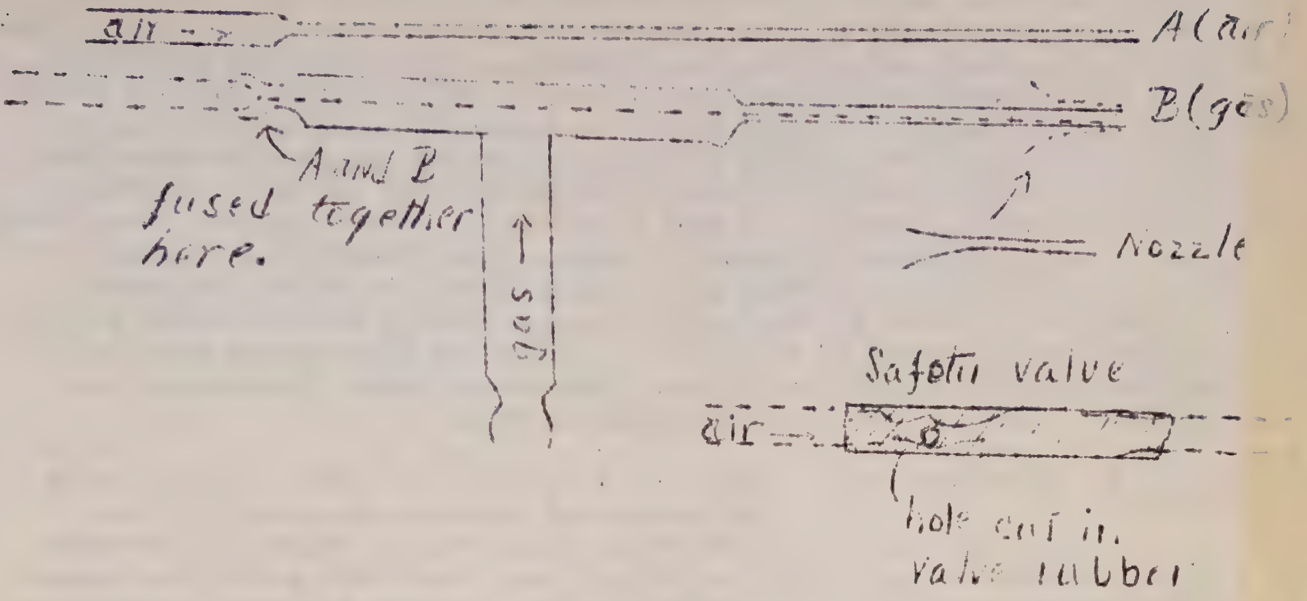
To prevent mold, not more than 0.1 percent Moldex is added. Food is prepared in the usual manner. When cool seed with yeast.

Morgan, L. V. Method for reattaching food when loosened from the culture bottle.

Food that has been loosened in a bottle during the period when counts are to be made can be reattached to the bottle with agar agar. Dissolve agar in water by boiling in a large pointed kitchen spoon. Allow it to cool sufficiently to be harmless to the fly larvae. Before it hardens have the loosened food medium in the bottle to be treated pushed a little away from theside of the bottle with a broad knife. Tip the bottle slightly and pour the melted agar from the tip of the spoon down the side of the bottle into the space made between the food and the bottle. The agar will fill the space and spread on the bottom of the bottle. Until the agar hardens keep the bottle tipped to prevent spread of any excess of melted agar over the suface of the food.

Pilkington, R. W. Construction of injection needles.

Construction of constricted needles is made much simpler by the use of a minute air-and gas blow-lamp formed of two concentric glass capillary tubes. To protect the inner (air) tube from becoming sealed up in the flame a small glass nozzle should be slipped over the end. - This micro-blowlamp can give an extremely small flame, and-which is most important-a sharply pointed one. An additional advantage is that with a strong flow of air the region around the flame is cooled by the draught, and the heating effect can be thus localized to aremarkable degree. - The blow-lamp is most conveniently used underneath a binocular dissecting microscope, with a black paper behing the flame to show it up distinctly. - In cases where the pressure of the air-supply is inconveniently high, and a slight movement of the tap will easily extinguish the flame, a piece of valve-rubber is inserted in the air-lead. The rubber should have a hole cut in it on one side; this forms a simple safety-valve if the tube is placed over a suitable shaped piece of glass tubing. - Needles can conveniently be sharpened on a rotating glass rod spread thinly with Rolls Razor strop paste. The paste which enters the tip of the needle can be removed by passing xylol through the needle.



Pilkington, R.W. Technique for the direct observation of live pupae.

With a suitable needle pupae can be removed fr the puparia as soon as the pupal skin has ha sufficiently, i.e. about 18 hours after pupa or rather less at 25° C. An ordinary needle set in a wooden handle is ground down flat in a grindstone. The needle should allowed to get red-hot during the grinding, as it can then be bent into a s shape simply by raising the handle away from the grindstone during the grin In addition, the heat serves to fuse the rough edges, and removes any jagged which might tear the skin of the pupa. - The tip of the needle is pushed th the pupa-case, the pupa being held securely by the posterior end. The hand the needle is then pressed gently downwards, the flat underside of the need ing on the pupa case. This causes the case to split towards the handle of needle. The operation is repeated until sufficient rings have been split; can then easily be removed by rolling the pupa over while one side of the s ring is held firmly on the operation plate with a second needle. - Each pup then placed on a glass slide and surrounded by a piece of filter paper in w hole slightly larger than the pupa has been cut. The posterior end of the should be in contact with the filter paper. A thin light cover-slip is the ed on top of the pupa, and the slide is placed in the incubator where some ment should be made to keep the filter paper moist. - For periodic observat der the microscope two or three drops of water should be run in under the c glass with a pipette, so that the pupa is completely immersed. The water s be dried off again with filter paper when the observation is complete. Pup be grown under these conditions for two or three days, and several have rea the adult stage and emerged from the pupal skin. By means of illumination the pupa with a well focussed pointalite bulb it is possible to watch the d ment of the wings, head, and such internal organs as are not obscured by th opaque fat body, and the bristles can be seen before the start of pigmentat

Spencer, W.P. Shipping Drosophila.

Aluminum tubing, ½ inch outside diameter, .0 wall, is cut into 3½ inch lengths. The usua meal-molasses of bannana-agar culture medium poured to a depth of 3/4 inch into a beaker or other convenient container a many of the aluminum tubes as will go into the container are set on end in medium. If a container with a tight cover is used, and moldex added to th ium, such a supply of tubes may be set away and kept for weeks in the refri To mail drosophila, a tube is broken out of the agar medium, a small cork i in the end of the tube containing the medium, flies shaken into the tube by use of a funnel, or etherized flies dropped into the tube. A cotton stopper is inserted. To make sure that flies do not stick to the aluminum wall we line the tube with a cylinder of paper; this ensures delivery without any of the flies sticking. These tubes may be labeled with India ink. Two such tubes containing flies can be sent in an air mail letter without further packing to any point in the U. S. for the usual .06¢ postage. Over 200 flies of one of the smaller species were sent to us from the Cal. Tech. laboratory in one of these tubes by ordinary mail service. My wife collected flies in Rio de Janeiro, Brazil, and sent them through successfully in these tubes by air mail letter, a distance of some 6000 miles. This method of shipping makes possible the use of air mail service for transit at small expense, and should prove particularly valuable to field collectors, mailing material back to laboratories. The aluminum tubing of this or other sizes may be purchased from the Aluminum Co. of America, 2210 Harvard Ave., Cleveland, Ohio. The cost for the tubes described above is approximately .01½¢ per tube.

Stalker, H. D. Substitutes for shell vials. Due to the cost and fragility of shell vials it has seemed advisable to search for some substitute to be used in making pair matings, and for ageing small numbers of flies. It has been found that small glass creamers, of one ounce capacity serve admirably. - They have the following advantages over shell vials: - (1) It is easier to transfer flies from one creamer to another than from one vial to another. (2) They are much easier to clean and pour than vials. (3) Due to the broad base, they rarely upset. (4) They can be closed with paper caps, thus saving time which would be used in making cotton stoppers. (5) They are much less fragile than vials. (6) Due to the wide mouth, eggs can be counted in them directly, and the larvae which hatch out can then be left in the creamer to go through to metamorphosis. - They have one disadvantage, namely they tend to dry out more quickly than vials, and if a large number of larvae are present, yeast suspension should be added from time to time. - The best results are obtained if the paper caps are punched with three or four small holes, and the food is poured to a depth of about 1.75 cm. - The one ounce creamers may be purchased from most resturant supply houses.. The Eagle Supply Co., 242 State St. Rochester, N. Y. sells them for $2.15 per gross. The paper caps may be purchased from Burr and Starkweather Co., 39 Mount Hope Ave., Rochester, N. Y. at a cost of $3.25 per 5000 caps, (No. 5/35 plain Tiplift).

Personal News

Things at present move so fast that a number of items listed in this column may be out of date. However it seems that in the upset circumstances in which we live, many will welcome even old and meager information about friends and colleagues. The majority of items are taken from letters which accompanied DIS material.

Africa

The activities in our Department have practically not been affected by the present war situation. Facilities are offered to build up a fairly good Drosophila laboratory. Keen interest is shown by students in the subject of genetics. The head of the medical school has arranged that Medical Science students be trained in genetics. Also, a first year course is compulsory for all medical students. But such a course is only forming an intergral part of the course in Zoology. It is not treated as a separate subject, with result that students from other Departments, e.g. Psychology, Sociology, etc. cannot benefit from Genetics lectures and practicals given. Information as to the present position of Genetics in American and other Universityies will be gratefully received. - Reprints from other workers are urgently needed, including those pertaining to classification of Drosophilids. We gratefully acknowledge the assistance of Prof. Sturtevant in this connection. (G. Eloff, April 16, 1940).

Europe, Great Britain

We are trying to keep genetics going in this country; indeed this ye
have as many as four research students all doing different things, namely
Primula heterostyly; (2) Some Neurospora breeding work; (3) Neutron effec
Tradescantia pollen-grain chromosomes; (4) Dosage-intensity relation in X
Tradescantia chromosomes. They each have additional experiments to fill
when the plants are in their off-season. (D.G. Catcheside, May 1, 1940).

As you will see from the directory enclosed, Drosophila work is in v
tive progress here. I hope shortly to have a fifth person working at it.
of us however gives all our time to Drosophila, as we all keep some plant
too. I was more under the impression that DIS had given me up. I did no
a copy of DIS-12. I realize that I had not sent any material for it, but
call came soon after the outbreak of war, it was not easy for us to assmel
material. (K. Mather, April 16, 1940).

The department is continuing in a comparatively flourishing manner.
live in our Animal House and with the exception of part of Chemistry, are
people belonging to the College still working officially in London. That
or Haldane is doing this seems at last to have been recognized by the auth
- The Drosophila work has never been curtailed and the mouse and rat stock
being reduced to a minimum, are being expanded in expectation of the rumor
turn of the College in the autumn. - We have three new colleagues, whose
work has been stopped by the war: Dr. Kalmus from the Zoology Department,
Callaway from the Imperial College of Science and Technology, and Mr. Renc
had only left our Drosophila work a year before to work for the Government
breeding. - During the last two terms we gave a course in Genetics at Birk
the only College of the University working in London, and in March Profess
also visited and lectured at Groningen and Amsterdam. (Helen Spurway, April
1940).

As you will see, we have so far been able to carry on with our work w
much interruption. At present I have two students, and I hope next year t
some more. I think there is a large field for developmental work on the I
phila pupa, and I hope to develop it. (C.H. Waddington, April 16, 1940).

Eurpoe, Italy

Since January I have been able to take up again my work here which pr
now well. - Timofeeff was in Rome at the end of March for delivering a con
at the Instituto di Sanita Pubblica, and afterwards he stayed here for a f
His work has not been disturbed so far by the state of war and he is conti
his work as usual. (A. Buzzati-Traverso, May 2, 1940).

Europe, Norway

Through the State Department Dunn received the following message from
" Inform Dunn Mohrs well".

Europe, Switzerland

Since Switzerland is one of the few European countries which at prese
has normal means of communication with all other countries, I am quite wil
offer to my colleagues in the warring countries my services as an intermed
scientific affairs. (E. Hadorn).

America, U.S.A.

After September 1, the address of C.W. and E.B. Clancy will be: Depar
Zoology, University of Oregon, Eugene, Oregon.

NOTES AND NEWS

Research Notes

Anderson, Ray The effects of Minutes on Bristle Number.

In checking the effects of various Minutes on the expression of the H^{32} gene, it has been found that the Minutes themselves frequently lack various bristles, especially the postverticals Preliminary results show M(3)w and M(3)Fla to be more extreme in bristle sence than $M(2)1^{2}$. In compound with H^{32} the Minutes act to decrease mea bristle number, with M(3)w and M(3)Fla again more extreme than $M(2)1^{2}$. expression of the Hairless and Minute genes in compound are greater than be expected on the basis of mere additive effects.

Brehme, Katherine S. Classification of the larvae of body color and bristle color mutants.

Certain of the mutations affecting adu body color or bristle color may be use as markers for classifying 3rd instar larvae, and are therefore useful in transplantation, physiological experim or the preparation of salivary chromosome slides. It has previously been reported (Brehme 1937) that the mou parts of larvae homozygous for y are golden to golden brown and are clas in the living larva as early as the 1st instar. The following yellow al have mouth-parts light enough in color to be readily classifiable: y^{1S}, y^{3d}, y^{4}, y^{6}, y^{td}. The following cannot be classified with certainty in instar: y^{2}, y^{34c}, y^{v2}; however, y^{3P} is classifiable with some difficult

The mouth-parts of stw^{3} are straw-colored at the basal prongs, and what lighter than wild type at the vertical plate; this is classifiable living 3rd instar larva, and with some difficulty in the 1st instar. Th prongs of stw^{3} are light, but classification in the 3rd instar is diffic

The tan alleles, t, t^{2} and t^{3}, are recognizable in the dissected 3r larva by slightly lighter basal prongs than those of the wild type; this ference cannot be used for classification of the living larva. The mout of su-t t are wild type. The obony alleles have blacker mouth-parts tha type throughout the larval stage, but the difference is too slight to be able in living larvae. The mouth-parts of T(1;2)Bld and of b are wild t

The ebony alleles, e, e^{4}, e^{11} and e^{s} are readily classifiable throu the entire larval period by means of the sooty color of the sclerotizati around the posterior spiracles, and, in the 3rd instar, around the anter spiracles as well. This sclerotization is clear yellow in the wild type Darkening of the spiracle sheath has not been observed in any other muta

Buzzati-Traverso, A. An extreme case of sex-ratio in D. bilineata.

A fertilized female caught in natur Pavia, Italy has given just females Such flies outcrossed to normal mal give just females, which show a gre fertility. Studios are under way to find the origin of such aberrant se

Curry, V. S. The mutant dwarf-24F, 2-13.5±, which is included in two overlapping deficiencies.

Homozygous dwarf-24F (dw-24F) is a fly, with dark small eyes, bloated abdomen, and slightly drooping wing It was found to be present in the l cg/Cy, al^{2} lt^{3} L^{4} stock, to the lef l(2)cg, and included in both Df(2)M Df(2)MC. Cytological analysis of these two deficiencies places it tonta in salivary section 24F. (Schultz)

It was reported previously (DIS-12;46) that Df(2)MB included l(2)cg Df(2)MC did not. This was an error due to misinterpretation of the results of crosses to the 1(2)cg stock which were made before the discovery of dw-24F. Since dw-24F/Df-Minute is relatively inviable, none survived in the cross to Df(2)MB, and it was concluded that the deficiency included the lethal. A few did survive in the cross to Df(2)MC, (though the phenotypic effects of dw-24F were not then recognized), indicating that this deficiency did not include the lethal. Retesting after the presence of dw-24F in the 1(2)cg stock was discovered has shown that dw-24F is included in both deficiencies, and 1(2)cg is included in neither.

Curry, V. S. Correction for Df(2)MB

Df(2)MB does not include the locus 1(2)cg, but does include dw-24F. (See above).

Curry, V. S. New data on the locus of lightoid.

The locus of lightoid (ltd) in 2 was previously placed at 55.0, to the left of the sp-a. Counts with ltd en/M(2)S2, place it 1. ⌿ to the right of the M(2)52 deficiency, hence to the right of the spindle attachment, ltd is therefore to the right of msf, since it is not included in a deficiency covering stw-msf.

Curry, V. S. Rotated genetalia in the mutant engrailed.

The mutant engrailed, in addition to previously described effects, produces rotated and malformed genetalia in ♂♂. The ♂♂ with extreme manifestation of this effect are sterile, hence there is selection for modifiers suppressing it in homozygous stocks. For example, the stock "blo er", which has been kept homozygous for a number of years, shows the effect so little that it had not been detected, though an occasional ♂ shows the extreme rotation. Homozygous engrailed from balanced stocks give it in much higher frequency; while among homozygotes recently extracted from a cross-over experiment (allowing for relatively complete change of genetic constitution) all the ♂♂ had rotated genitalia, some with the penis entirely lacking, and all were sterile. A heterozygous stock was made and from this homozygous ♂♂ capable of breeding were obtained later. A homozygous stock is now maintained by means of transferring large numbers of flies. (The heterozygous stock is kept for safety.)

Comparison of this homozygous stock and the heterozygous stock, after about two months, gives striking evidence of the selection for suppressing modifiers where there is necessarily a selection for fertility. The heterozygous stock gives homozygous all of which show strong rotation; while in the homozygous stock, more than half show the strong rotation (90° or more) and the remainder show varying degrees of asymmetry or rotation approaching normal, or slight malformation without discernible rotation.

There is some evidence that the extra sex-comb of the engrailed ♂♂ is heavier in those showing the more extreme effect on the genitalia, but this has not been checked by accurate measurements.

Green, M. M. A spontaneous mutation of the gene vg to a weaker allele.

An apparently spontaneous mutation of the gene vg to a lesser penetrant allele, tentatively called vg^{nG}, was observed in 1 ♂ and 4 ♀ in a vg stock inbred in mass culture. Homozygous vg^{nG} exhibits wild type wings. vg/vg^{nG} exhibits notched wings in 100% of the cases studied thus far. Occasionally marginal scalloping occurs. $vg^{nG}/Df(2)vg^{D}$ exhibits a somewhat more extreme scalloping than the vg/vg^{nG} phenotype. No decrease in the haltere size or scutellar bristle arrangement has been detected in the compounds thus far tested. Tentatively this mutant is considered as similar to vg^{n}.

Kalmus, Hans Resistance to desiccation of some body color mutants in Drosophila.

Differences in resistance to desicc have been found in mutants of the fo species *D. melanogaster*, *simulans*, *pseudoobscura*, and *subobscura*. The methods employed were measuring the death rate and loss in weight, presumed to be loss in water, by means of a torsion balance. In all four species the y mutant is less resistant to desiccation than normal. In *melanogaster*, e and black increase the resistance to desiccation.

Kikkawa, H. Chemically detective methods of the substance concerning eye, egg and other colors in insects.

Recently I have discovered that the substance which has the v$^+$ property well found in Drosophila and in othe insects can be detected by the so-ca Ehrlich's diazo reaction technique, namely; Reagent I 1.75% HCl containing 0.5% sulphanilic acid and Reagent II 0.5% sodium nitrite ($NaNO_2$).

Mix the reagents in the ratio 40(I) to 1(II) when used. Add the mi the solution to be tested to which a few drops of concentrated ammonia i The solution shows a beautiful scarlet color when the cn substance is The substnace showing above reaction may be called the + chromogen, for this substance is analogous to Sachs' color-substance (Hoppe-Seyler's 242, 1936) or to Weiss' urochromogen (Biochem. Z 133, 1922), both of whi assumed to be the derivatives of tryptophane. But there are some differ in chemical properties among these substances. It seems difficult to ob the substance in crystalline forms with the ordinary method. Ehrlich's reaction as well as its modified method; Pauly's one, is not specific to + chromogen and its analogous substances, because imidazol derivatives histidine and p-oxybenzene derivatives like tyrosine show a similar reac But there are certain evidences that Ehrlich's diazo reaction of the wa soluble substances of insects can not be attributed to the presence of h or of tyrosine. The extract of v or cn mutant of Drosophila does not sh reaction, but that of wild, bw or of other eye-color mutants containing substance does show it. Similarly in Bombyx, the body-fluid of wild pu the extracts which contain no or little cn$^+$ substance give almost nega sults.

Kynurenine shown by Butenandt et al to be one of the v$^+$ substance be found in a mutant of Bombyx called white-1, which lacks the w-1$^+$ (W) and shows a maternal ingeritance. I have succeeded in obtaining crysta kynurenine-sulphate from the extract of eggs of this mutant, but kynure does not show Ehrlich's diazo reaction. The evidence that kynurenine i formed to the + chromogen showing Ehrlich's diazo reaction has been der the following experiments. When the v, bw larvae of Drosophila are tre food containing kynurenine, the body-fluid does not show the kynurenine (see below), but gives Ehrlich's diazo reaction. Similarly when the wh eggs of Bombyx are colored grayly by an implanted ovary having the w-1$^+$ the extract of those eggs shows both Ehrlich's diazo reaction and the k reaction, though lightly. Besides, when the + chromogen is heated with one can smell a jasmine-like aroma of o-aminoacetophenon, as in the cas kynurenine. Furthermore, one can detect the presence of anthranilic ac the resolutive solution of the + chromogen. Those finding indicate tha chromogen is composed of a substance similar to kynurenine.

A specific and very keen method of detecting kynurenine has been f Otani and Honda who are collaborators of Prof. Kotake (Osaka-Izikaizass 1938). Ehrlich's reagent, 15% HCl containing 2% p-dimethylaminobenzald (sometimes 10% HCl is more adequate)is added to the solution to be test which 3% hydrogen peroxide of the amount half of the reagent is further The solution is then heated 10-20 minutes in a water bath of ca. 70°C.

solution shows a beautiful violet color if kynurenine, anthranilic acid or aminoacetophenon is present. The latter two substances may be distinguished from kynurenine by shaking the solution with butanol for several times, because they are transsolved into butanol. The grade of keenness of the above method is increased by leaving the mixed solution at a room temperature for 14-20 hours, instead of being heated.

As far as my examinations go, only the white-1 mutant of Bombyx shows this reaction clearly. The cinnabar of Drosophila and the orange and ivory mutants of Habrobracon seem to belong to this category, but are not conclusive at present. Thus we can obtain at least the following system in connection with the formation of eye and egg colors, etc.: Compound tryptophane, Kynurenine ⌿ chromogen ⌿ chrome (eye-color substance, egg-color substance, etc.). It is needless to say that there are several intermediate forms in the above processes. For example, alpha-oxytryptophane and prokynurenine are to be located between tryptophane and kynurenine. Probably enzyme actions (resolutive and synthetic) or physiological conditions take part in the above transformation. It seems very plausible that these enzyme actions or physiological conditions are controlled by the genes such as v^+, cn^+ and $w\text{-}1^+$. This assumption was already suggested by Butenandt et al. It is to be noticed here that the above system concerning the color-formation may be operated similarly not only in Drosophila and Bombyx, but also in insects such as Ephestia, Habrobracon, Calliphora, and even in various other arthropods.

Milani, R. Two new eye-shape mutant alleles in D. melano.

From a culture with a number of eye-color mutants of the X-chromosome, a new mutation has been isolated which produces the total disappearance of the eyes. A few bristles placed on a line are present where the eye is lacking, in its most extreme manifestation.

Such mutation shows a rather large variability: sometimes the antennae are fused, sometimes just a few ommatides are present, sometimes one eye is normal while the other one is transformed into two small ones. Such mutation is placed in the second chromosome; the flies carrying such mutation have a good fertility; the viability of mature pupae is lowered (30% at 17° C., 99% at 30° C.). The mutation has its best manifestation at 20° C., while at higher or lower temperatures a number of rudimental eyes are produced.

A less extreme allele of such mutation has been found. It can be easily distinguished from a less expressed form of the original mutation by the total disappearance of the ocelli in such allele.

Morgan, L. V. Allelomorphism of Cat and spa, Mutants in chromosome 4 of D. melanogaster.

The loci of Belgovsky's dominant mutation Cataract (Cat) and of the recessive sparkling (spa) in chromosome 4 of Drosophila melanogaster have not been determined, but the mutants are found to be allelomorphs. Cat is homozygous lethal. The posterior third (or half) of the ye of a fly heterozygous for Cat is decidedly rough and the arrangement of facets is confused. The eye in homozygous spa female is somewhat bulging and the entire surface is more or less rough and sparkling. Both characters are variable; spa is more pronounced when flies are raised at 19° than at 25° and its manifestation is suppressed in the presence of a Y-chromosome (in XXY and XY). Among Cat/spa flies:--the eyes of XXY females show Cat and not spa (Y present); eyes of XX females are bulging and show the rough and sparkling effect of spa and also posteriorly the effect of Cat; eyes of XY males show Cat but not spa (Y present); eyes of XO males, like those of XX females, show both Cat and spa. The relation of heterochromatin to the manifestation of spa is being studied.

Parshley, Elsa M. Development of the eye of Minute (3) Fla.

Larvae from crosses of ca/ca ♀♀ by Fla/ca ♂♂ on a background of Florid type were cultured at 25° C in 7 cm. Petri dishes, 30 larvae to a dish, on a cornmeal-agar-molasses medium with brewer's yeast. Their age was known within 2 hours from hatching from the e At 48, 72, 96 and 122 hours, the larvae were classified by Malpighian tube and the optic disks dissected out in physiological saline solution. The were flattened on a slide by means of a thin film of larval grease, and lucida outlines drawn; planimeter measurements were then made of each dr It was found that the 48-hour and 72-hour wild type disks were more than as large as the Minute disks; at 96 hours, the wild type disks were appr ly 1.5 times as large as the Minute. Preliminary measurements of 122-ho ute disks indicate that they continue to increase in size after the time puparium formation of the wild type. - Facet counts of adult eyes show t there is no significant difference between wild type and M(3)Fla (claret 731.2 facets, Minute ♀♀, 741.8; claret ♂♂, 676.1 facets, Minute ♂♂, 68 Superimposed drawings of blocks of 9 facets each from corresponding part Minute and wild type eyes showed no difference in facet size. Tibia mea ments of the same flies as those used for eye measurements show that the adult, taking tibia length as an index of body size, is slightly smaller the wild type. - It is concluded that the M(3)Fla factor affects growth optic disk in such a way that the area of the Minute disk by 48 hours is than half that of the wild type; growth of the Minute disk continues dur interval between puparium formation of the wild type and Minute larvae; final product, the imaginal eye, is the same in facet number and facet s both genotypes. Body size of the Minute fly is smaller than the wild ty Minute eyes are therefore larger in proportion to body size than the wil eyes.

Philip, Ursula The chromosomes of D. subobscura.

The mitotic complement consists of f pairs of rod shaped and one pair of do shaped chromosomes. The X and Y chrom somes, which are indistinguishable f each other, are the largest of the s Whereas the secondary pairing at metaphase is complete in the autosomes, the proximal portions of the sex chromosomes pair, the distal portions re each other.

The nucleus of the salivary gland cell shows five long and one very chromosome. The number of strands thus corresponds to the number of chr somes. The general morphology of the nucleus shows some interesting fea The chromosomes are held together in a chromocenter which however is so that the strands are liable to break away. This means a relative lack o heterochromatin. We have not yet identified the strands with the linkag One of the chromosomes provisionally labelled IV has an intercalary segm heterochromatin, which in favorable preparations can be observed to pair the chromocenter. Chromosome 2 has a proximal and a distal segment of he chromatin and is often seen as a loop, both ends fixed in the chromocent

The Y chromosome contains a euchromatic section of at least 15 band

The stocks in this laboratory contain many aberrations.

We have so far been unable to find a single stock which could be us standard, with all the chromosomes homozygous. The aberrations are most inversions, but two small deficiencies and one small duplication have be as well. Double inversions of three types have been seen.

The chromosomes are in process of being mapped. No racial groups h established, because the material, though derived from the inbreeding of from several English localities, has been extensively crossed.

1 (1) Median deficiency 10 bands distal inversion 30 bands
2 ----------
3 Distal two overlapping inversions 70 bands
4 Distal single inversion 60 bands
Distal deficiency inside overlapping inversion 2 bands
Distal duplication 3 bands
5 Median two included inversions pairing in the middle portion leaving a loop at each end (60 bands)
Two adjacent independent inversions 80 bands.

Russell, E. S. Comparison of benign and lethal tumors in D. melanogaster.

Transplantation of benign tumors from five different strands and of the "lethal" l(1)7 tumors into normal Canton-S and Oregon-R larvae have produced "takes" with all types, the tumors proliferating until pupation of the host and normal adults developing containing the injected tumors. Histological studies of the tumors show that all of them, including the l(1)7 tumors, are very similar in structure, spherical masses of tiny cells surrounded by hollow spheres of melanin. They arise in general in the same organs, at the same time, and their history is the same. These data indicate that the l(1)7 tumors are no more malignant than the benign types, and suggest there may be another cause of death in the l(1)7 larvae. An abnormality of the mid-gut, practically obliterating the lumen, has been found in these larvae, and it may be the cause of death. Experiments are going on at present to determine the origin and manner of action of this abnormality. The inheritance of tu36a, a benign, is also being studied in detail.

Schultz, J. Change in temperature of the stock room at Pasadena--an opportunity for experiment.

The stock room at Pasadena has been maintained at a temperature of 19° ± 1° for the past five years. Stocks are transferred at this temperature about every three weeks, the continuation being from small samples. Under these conditions there is of course a selection in each of the 812 strains for modifiers providing an optimum viability, etc. It is now intended, in the interests of reducing the labor of stock maintenance, to lower the temperature to 17°. For each of the stocks this will bring about an "evolutionary" change in which by selection as time progresses the genetic structure of the population will be changed. An opportunity is thus offered for many diverse types of experiment. We suggest therefore that anyone interested in making use of this opportunity, and desirous, for purposes of later comparison, of studying the present structure of any given stock, communicate with us within sixty days.

Schultz, J. Confluens a tandem duplication of the Notch region.

The mutant Confluens, located by Gottschewski in the Notch region, was found by him to neutralize the Notch phenotype, and to give crossovers with split (DIS-4: 7, 14, 16; DIS-8:12). Current reinvestigation of this case has shown that: (1) Confluens is associated with a tandem duplication of the Bar type, for the bands 3C$_5$-3D$_6$ (approximately); (2) that the Confluens phenotype (dominant, not as previously described recessive) is the result of duplication of the 3C$_7$ band, deficiency for which gives Notch (evidence from crossover experiments between Notch and

Confluens); (3) reversion to wild type occurs in homozygous Confluens; (4) pairing of the duplicate sections may be quite close and so simulate "swelling" of the region such as has probably confused Hager's study of Bar types; (5) X-radiation of Confluens has given a series of derivative of which show the characteristics of the mutants Abruptex (Ax) and split associated with breakages in this region. Their properties, as well as Ax (itself probably a shorter tandem duplication like Hw) are under stud

Timofeeff-Ressovsky, H. A. and N. W. On the determination of the number of individuals in populations of Drosophila. In two previous notes (DIS-11) the determination of the spatial distr tion and of the "radius of activit Drosophila-individuals was describ The method used consisted essentia subdividing a certain territory (" mental field" of an area of 1-5 he into equal squares (of 10-30 m side-length), in the centers of which foo bottles were placed; once or twice a day during a period of 1-2 weeks th caught in these food-bottles were counted and registered; for determinin "radius of activity" - a certain number (1000-5000) of "marked" (by one non-deleterious mutations) flies were let out in the center of the "expe field", which then were caught and registered in the bottles of the surr squares during the next two weeks, thus showing the extension of the dis tion-area of Drosophila-individuals. Essentially the same method can be for determining the approximate number of individuals per isolated colon Drosophila-species, or on a certain limited area. If the numbers of "ma flies which were let out in the center of a certain area (larger than th "radius of activity"), and of the marked and non-marked flies caught on area are known, then the total number of the non-marked (wild) individua the area can be easily calculated with a certain approximation (consider sources of error, due to possible differential mortality and catchabilit Such determinations of the number of individuals were repeated several t during a breeding season in an isolated small population of Drosophila m gaster and Drosophila funebris (showing maximum values of about 35000 fo bris in July-August, and of over 60000 for melanogaster in August-Septem and on a garden-area for melanogaster and species of the "obscura"-group ing peak values of about 5000 for melanogaster in July, and of about 150 throughout the whole summer for "obscura"). This method can be used in ferent modifications, and in connection with different problems concerni dynamics of populations.

Technical Notes

Beadle, G. W. A rapid method for removing pupal cases. In observing the development of Dr pupae it is often an advantage to the pupal case. This has been do number of workers, for example, R and Bodenstein. In injecting sol such as those containing eye-color hormones, into pupae it is desirable move the pupal cases for two reasons: (1) to reduce breakage of pipette (2) to reduce internal pressure in the pupae. Since the method of disso away the case requires great care and is time consuming, an easier and rapid method has been developed. Pupae of the desired age are lined up edge of a glass slide cut down to fit in a shell vial. We use slides ab 2½ x 3/4 inches - these fit conveniently into 8 dram shell vials. About 20 pupae can be put on one slide. Duco waterproof cement is run down th of the slide and the pupae quickly moved into this with fine forceps. P are lined up transversely to the long axis of the slide, ventral side do After drying, a dorsal-lateral strip of the pupal case running the entir of the pupa is "shaved" off with a scalpel made from a chip of a double-safety razor blade. Such razor blade chips can be mounted in a small pin vise or dissecting needle holder chuck. Care must be taken not to injure the wing during this process, but with practice the cut can be made quickly and in a single operation, starting at the posterior end of one emergence suture. With a sharp pointed pair of watchmakers' forceps the cases can be peeled away from the pupae. If desired the pupae can then be lifted out of the cases, but for injection operations it is convenient to leave them in the opened cases. Injections are made into the anterior dorso-lateral portion of the abdomen with the pipette directed toward the posterior end of the pupae. After injection the entire slide is put in a shell vial with moist filter paper. Pupae prepared in this way develop normally although not all of them are able to extricate themselves from the pupal membranes at emergence time.

Gordon, Cecil Nomenclature

The term 'spontaneous' as opposed to 'due to irradiation' is ambiguous for it draws no distinction between newly mutated genes and those probably present in the original population from which the flies have been drawn. The following is suggested. Spon. (S) - Spontaneous from an established stock and hence likely to have arisen recently. Spon.(P) - Spontaneous, but from stocks not long in laboratory and hence probably present in original population.

Kalmus, Hans Skimmed milk as a culture medium for Drosophila.

Drosophila melanogaster, *virilis*, *subobscura*, *buskii*, and probably other species can be bred on skimmed milk. The method is as follows: Cellu-cotton, mentioned by Spencer, (DIS-8) is pressed into vials or bottles which are stoppered and sterilized. They are filled with fresh skimmed mild and put into the incubator for 24 hours. After this period the skimmed milk has turned sour and vials and bottles are ready for the flies to be put in. The four mentioned species grow satisfactorily on this medium, and the number of offspring of 5 females cultured in a vial were: *melanogaster* 62 ♂, 77 ♀ ; *virilis* 41 ♂, 29 ♀ ; *subobscura* 60 flies; *buskii* 83 ♂, 78 ♀ ; from bottles the numbers were of course, larger. For example, for *virilis* 192 ♂, 203 ♀. The time of development seems to be shorter than on the ordinary food medium, but this may not be significant. The medium apparently contains very little yeast and its flora consists mainly of *Streptococcus thermophilous*, *Lactobacillus bulgarious*, and similar milk bacteria.

This method, where the medium is more simply prepared than the ordinary food, is slightly quicker.

La Cour, L. F. Acetic Orcein for Salivary Chromosomes.

The use of this new combined staining fixative gives more selective staining than acetic carmine. The methods employed are the same, with the new formula, which consists of 0.5% orcein (elastin type) dissolved in hot 45% acetic acid and filtered when cold.

This acetic orcein shows the gene structure of the chromocenter as well as the ordinary bands in the salivary gland chromosomes of many species of Drosophila more clearly than acetocarmine. In particular it reveals the gene thread connection between the chromocenter and various bodies in the nucleolus. In D. funebris it appears that there were several of these bodies, which possibly act as nucleolar organizers since they are connected by separate threads with the chromocenter.

Lefevre, George An electron bombardment of D. males. An attempt to bombard Drosophila melanogaster males with electrons has been made in the hope that chromosomal rearrangements might be produced. A very simple apparatus is sufficient. Two tungsten electrodes are sealed into the ends of a glass tube about 4 inches long and 3/4 inches in diameter. From the middle a side-tube extends through which the flies may be introduced into the chamber, and which can be connected to a vacuum pump. The electrodes are connected to an induction coil, and current is furnished by three storage batteries. A vacuum of about 1/1000 mm. Hg. is required for a maximum flow of electrons, and the flies are able to survive such a low pressure for several minutes in most cases. Minature males were introduced into the tube, which was then evacuated, and the current turned on for 1/2 to 2 minutes. Fifteen flies have survived this treatment, but only one male, mated to an attached-X yellow female, has produced progeny. No visible mutants have been observed. Further efforts are being made.

Poulson, D. F. and Power, M. E. The application of Bodian's silver impregnation method to Drosophila. For studies on neurology and the development of the nerve fibers in Drosophila, modifications of Bodian's method give excellent results, both in early stages and in adults. Since the method produces a brilliant picture of details (except within the nucleus) preparations of this kind are useful for much more than the study of nervous and sensory structures. For instance, cytoplasmic differentiation is visible in the salivary gland cells within 8 hours of fertilization of the egg. The following procedures have been used: - Eggs and Larvae--Fixation in formol-alcohol-acetic(F.A.A., 5:15:1), or Petrunkevitch's paranitrophenol to which an equal volumen of 10% formalin has been added, for 24 hours. Proceed as usual in preparation of sections of eggs or larvae. Cut at 7-10 u. Mount on carefully cleaned slides (see below). Before impregnation it is well to harden the mounted sections and albumen by allowing the slides to stand overnight in 95% alcohol. Impregnate in 1% protargol solution (aqueous) at 38° C. for 48-96 hours. Copper shot should be placed in the bottom of dish in which this is done. Slides are then rinsed in water and the silver reduced for 10 minutes in a solution of 1 gm. hydroquinone and 5 gms. sodium sulfite per 100 cc. distilled water. Rinse three times in distilled water and place in 1% gold chloride solution for 15-20 minutes; rinse, then place in 2% oxalic acid for 15-20 minutes, controlling with a microscope until sections appear quite dark. Rinse with care in water and fix in 5% sodium thiosulfate for 10 minutes. Rinse gently 3 times in water and dehydrate slowly using a graded series of alcohols. This care is to prevent sections from coming loose after such prolonged hydration. Clear and mount as usual. - Adults--In preparing adult tissues for staining with the Bodian method entire flies or parts (in this instance, heads) are fixed in alcoholic Bouin's for one or two days. If the tissues float the dish containing them and the fixative is placed in a vacuum chamber. Alcohol, tuluol, and tissue-mat are used for dehydration, clearing and embedding. The slides are carefully cleaned in cleaning solution and ammoniacal alcohol to insure that the sections remain attached throughout the procedure. Adult nerve fibers are larger and require longer impregnation than that mentioned above. A 2% aqueous solution of protargol is used, and enough copper shot to cover the bottom of the dish is added. Impregnation is at 38° C. for 24-48 hours, after which time the slides are removed, rinsed in distilled water, reduced in the 1% hydroquinone-5% sodium sulfite solution, rinsed in three changes of distilled water, and returned to the oven in a fresh bath of protargol and copper for another 24-48 hours. At the end of this time the slides are removed and rinsed, reduced and washed as above. Then the slides are placed for 10-15 minutes in 1% gold chloride, rinsed, treated with 2% oxalic acid for 10 minutes, rinsed, and left in 5% sodium thiosulfate for 10 minutes. The slides are then carefully rinsed three times and run up and covered in the usual manner. Fresh solutions should be used for each batch of slides, except the gold chloride which may be re-used. - Appearance of structures--In the preparations nerve fibers, nerve endings, Golgi material and other cytoplasmic elements, muscle striations, and chitin are deep purplish black; the bulk of the cytoplasm is light purplish red, occasionally almost grey; nuclei and chromosomes very dark red; in dividing cells spindles as well as chromosomes are dark red. Some preparations may be darker and more purplish than others; occasionally paler than redder.

Ross, Elinor, and Russell, E. S. A satisfactory histological technique for D. larvae.

Fixation: Larvae are immersed in hot Carnoy's solution (60°) until straightened, pierced with a glass needle. Then they are passed through the following solutions:

Solution	Time
hot Carnoy's	30 minutes
cold Carnoy's	30 minutes
95% alcohol	rinse, then 1 hour
95% alcohol + eosin (stain for embedding)	1 hour
absolute alcohol	1 hour
xylol	until clear (1 hour +)

Embedding: The larvae are put through three changes of tissue-mat (56-58°) and imbedded in tissue-mat. They may be sectioned sagittally or transversely at 8 u. - Staining: Galigher's alum-hematoxylin for 90 seconds or less, counterstained with Triosin (.5% solution in 90% alcohol) for 20-45 seconds gives a clear delicate stain which is very good both for study and photography, especially where contrast with black material such as melanin is desired.

Personal and Laboratory News

(Editor will be glad to receive and to circulate information about colleagues in Europe and about working conditions in European laboratories)

Europe

France

L'Heritier is back at teaching and has facilities for research work. All his Drosophila cultures were lost. A collection of 12 stocks was sent to him by air mail.

Ephrussi, according to the information just received, he and his family are now with L'Heritier in Clermont-Ferrand.

Vandel is well and his work is going on.

Germany

Information received indicates that the work at Berlin-Dahlem and at Buch is going on normally.

Great Britain

John Innes Horticultural Institution

Drosophila work is still flourishing here though it has to be subordinated to other problems notably certain economic lines that we have taken up. All the people in our last directory are still here and have been joined by two newcomers. (K. Mather, December 26, 1940)

University College, London

The department has at last gone away from London; Dr. Gruneberg to Mount Vernon Hospital, Northwood, and Professor Haldane and the three Drosophila workers to the Rothamsted Experimental Station. Dr. Philip has been working at the John Innes Horticultural Institution since September 1939. University College has been very gravely damaged but we have escaped so far with a few smashed windows and an erratic and scanty supply of gas. However we have kept all our stocks and even collected a fair amount of data by means of shifts like cooking at home or at the John Innes. Two members of the department have been bombed - one out of a house, the other in a public shelter - and one has been forced to wash his baby in Highgate pond water. - At Rothamsted we have been made very welcome and are going to try to carry on throughout the war. (H. Spurway).

Cambridge: Christ's College

We are getting on fairly well here, with practically no bombing in the to so far. As a fair number of people from the lab have been taken away to other jobs, those of us who are left are rather overburdened with teaching, and what research we can do is directed as far as possible towards practical ends, so that Drosophila is rather suffering. (C. H. Waddington).

Edinburgh, Scotland

The conditions of work here are really very nice. The institute is working very efficiently and one would never guess that there is a war. (Slizyniski, B.)

Italy

Research work with Drosophila at the Pavia Institute has stopped. Only one member of the staff and one student are present--none of them working with Drosophila.

Norway

It has been learned from reliable source that Dr. Mohr and his family were in good health early in November. - A letter written in January was received indicating that Mohr is well.

Switzerland

Work was interrupted several times by military service.